HYPERTENSION

Humoral and Neurogenic Factors

CIBA FOUNDATION SYMPOSIUM

ON

HYPERTENSION

Humoral and Neurogenic Factors

Editors for the Ciba Foundation

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PREFACE

This symposium on Hypertension had its origin in a suggestion by the late Professor H Rasmussen of Norway Just before his untimely death he proposed that a conference should be organized for discussion of hypertension from the epidemiological point of view As Professor Pickering mentions in his Opening Remarks this project has not been abandoned but it is proving difficult to arrange and in the meanwhile with Professor Pickering s invaluable help the symposium of which this volume contains the proceedings has been held as a complementary discussion to the one in view

A word about the Ciba Foundation must be added here by way of explanation to those to whom this book serves as an introduction to its activities It is an international centre established as an educational and scientific charity under the laws of England It owes its inception and support to its founder Ciba Ltd of Switzerland but is administered independently and exclusively by its distinguished British Trustees

The Foundation provides accommodation for scientific workers who visit London from abroad organizes and holds international symposia conducts (in conjunction with the Institut National d Hygiene) a post graduate medical exchange scheme between England and France arranges informal meetings for discussions awards an annual lectureship assists international congresses and other scientific societies is building up a library service in special fields and generally endeavours to give aid in all such matters as may promote international co operation in scientific research

Leading research workers from different countries and in different disciplines are invited to attend the symposia or colloquia The size of the groups is however very strictly

limited in *order* to obtain a free conversational manner of discussion—although the basic timetable of the programme is strictly observed The smallness of the groups necessarily means the exclusion of many other workers active and interested in the subjects discussed, and therefore the proceedings of these conferences are published and made available throughout the world

It is hoped that the papers and discussions in this book will prove not only informative and stimulating, but will also give to readers a sense of participation in an informal and friendly occasion

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CHAIRMAN'S OPENING REMARKS

G. W. PICKERING

I AM sure that the first thing you would like me to do is to express our appreciation to the Ciba Foundation for arranging this meeting and for allowing us to meet in this highly agreeable atmosphere to discuss these matters of common interest. But it is not only for that that I think we should thank its Director, Dr. Wolstenholme. He has arranged nearly all this Symposium and I have been astonished at his remarkable and detailed knowledge of the whole field of the subject about which we are talking.

There is no need, I think, to stress the importance of the problem of hypertension and its effects on the cardio-vascular system. The problem could have been approached from two points of view: from the functional point of view, or if you like, the point of view of applied physiology, which is the purpose of this Conference; or from the epidemiological point of view, and I have the Director's permission to mention that he has it in mind to have a future Symposium on this second aspect of the problem.

Twenty-two years ago, when I started to work on hypertension, having been trained as a physiologist and working in a laboratory which was given to the physiological approach, the laboratory of Lewis, it seemed to me that this would be a relatively simple problem to solve. All it needed was the application of the experimental method and physiological principles. It is rather sad to look at it again after twenty-two years and see that I have to count myself amongst those who do not know the answers to most of the questions which we ask. We know of course that it is possible to produce hypertension in the experimental animal by a variety of means. But I think it is only in that particular variety which

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NEURAL AND HUMORAL CONTROL OF BLOOD VESSELS

IRVINE H PAGE

THAT an international conference has been called to discuss neural and humoral controls of the circulation is proof enough of the station this topic has attained The field has developed in a truly international way such names as Abell, Dale Elliott Fourneau and Cannon occur among the early workers in this fruitful vineyard We who have come later have our reward now in the benefits of this present association and hospitality for which we thank the Trustees of the Ciba Foundation and the scientific perceptiveness of Dr Pickering and Dr Wolstenholme

I am told that I am not intended to document the topic but rather to express the views activities current interests and aspirations of our group at the Cleveland Clinic The aim in brief is to provide a setting for discussion rather than a manuscript to moulder in the archives

Those aspects of cardiovascular regulation with which I am most concerned can best be set in their place by visualizing the problem as a whole This I have tried to do by an octagonal diagram which indicates the controls of tissue perfusion function in a dynamic equilibrium This concept I regard as important because it seems that the problems we face are more realistically soluble in terms of altered equilibria than by any one of a variety of monistic approaches

For this reason we have been particularly occupied with the problem of vascular reactivity i e of the response to a vasoactive agent and the factors which modify this response This area of investigation has been too widely overlooked The fact is that the response of the tissue to a drug or procedure is at least as important as the nature and quantity of the

is produced by section of the carotid sinus and depressor nerves that we have even the remotest appreciation of what the mechanism of the hypertension actually is I believe it is still true to say that in the hypertension invented by Goldblatt, whom we are extremely sorry not to have with us here today, we do not know the answer, and I think perhaps the same is true concerning the hypertension that follows nephrectomy I say this, speaking at 4 15 p m on the 27th of July I hope that at a corresponding time on the 30th of July I shall be able to revise these opinions

It is not surprising, if we know so little about the mechanism of hypertension in the animal, that we know even less about it in man, even in those relatively well defined conditions in which the hypertension is associated with lesions in the kidney which can actually be seen under the microscope and with certain disorders of the endocrine glands which again are perfectly obvious Perhaps it is not so surprising that in that much more elusive and intangible phenomenon, essential hypertension, we should be even further in the dark So that this problem of hypertension provides a challenge to clinicians, biochemists, pharmacologists and physiologists and I take it that it is our purpose here and now to try and accept that challenge to pool our ideas and knowledge, and see how far we can get

some time to elicit chronic arterial hypertension by means of cerebral ischæmia in dogs Each new induction of ischæmia caused only a transient episode of hypertension eventually a point was reached where increased ischæmia was lethal However we went on to combine cerebral ischæmia with repetitive stimulation of the floor of the fourth ventricle—the latter was achieved by implanting a tantalum wire and subjecting the animal s head to an inductotherm at intervals after the operation—and the result was a persistent neuro genic hypertension of moderate severity which was abolished by section of the spinal cord at C6

The hypertension which follows section of the carotid sinus and aortic depressor nerves is similar The blood pressures of these animals are often increased to levels of 300 mm Hg and over the hypertension can be relieved by total sympa thectomy or abolished by high spinal cord section Increased cardiac output due to cardiac acceleration was once believed to be the major factor in causing this increase in arterial pressure However it now appears that the disequilibrium of sympathetic control extends to the arteries and arterioles since the hypertension persists after cardiac denervation when cardioacceleration is absent (McCubbin and Page 1951)

The responses of these dogs to tetraethylammonium chloride (TEAC) are of particular interest to me they demonstrate the concept of reactivity validate the view that sino aortic neurogenic hypertension is due to misplaced equilibrium of sympathetic vasomotor controls and may yield some insight into the problem of hypertension in human beings Briefly stated intravenous injection of standard test doses of TEAC causes in these animals deep briefly sustained, repetitive depressor responses whereas in normal dogs or in dogs with renal hypertension initial moderate depressor responses are followed by pressor responses to successive injections (Page and McCubbin 1951 1952a) The drug itself acts primarily by blockade of transmission through sympathetic ganglia the responses in the dog with neurogenic hypertension indicate as might be predicted that these

stimulus The technique used in most of our experiments consists in observing the changes which occur in an animal's arterial pressure after administration of a variety of vaso active drugs Obviously, various mechanisms can participate in these changes, such as cardiac output on the one hand or peripheral vasoconstriction on the other, when changes of

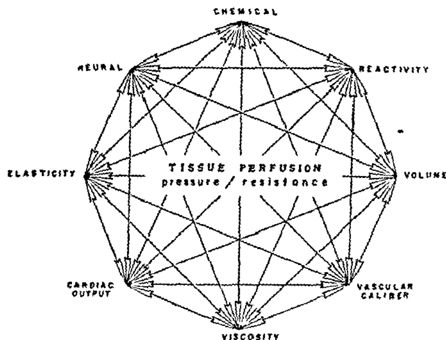


FIG 1 Interrelationship of various factors controlling tissue perfusion

reactivity can be shown to occur specific and complex methods of analysis must be used to determine their nature

Neural Factors

Neurogenic Hypertension As has been shown by electrical stimulation, certain areas of the brain influence the level of arterial pressure The significance of these acute responses remains obscure Because of our interest in the problem of chronic hypertension Dr Taylor and I (1951) attempted for

this form of clinical hypertension like the experimental disease will respond to sympathectomy

Reactivity and Neural Change Destruction of the spinal cord from C6 caudally if the animal or patient survives in relative well being sharply increases responses to test doses of noradrenaline adrenaline and barium chloride responsiveness to angiotonin is unaffected or only slightly enhanced sensitivity of the vagal cardio inhibitory reflex is greatly

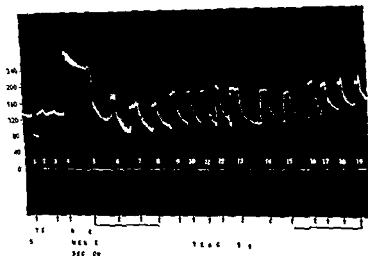


FIG 3 Response to TEAC before and after section of the buffer nerves

augmented In brief this form of neural ablation induces a definite pattern of reactivity change

Section of buffer nerves induces a different series of changes Thus as McCubbin and I (1952) showed section of the carotid sinus nerves, when preceded by section of the vagus depressor trunk and administration of TEAC greatly enhances the response to angiotonin responsiveness to noradrenaline serotonin and Pitressin are not proportionately increased sympathectomy prevents this effect of buffer nerve section

ganglia are transmitting a vast shower of vasopressor impulses, the responses are deep because these impulses have become the major determinants of the blood pressure level, they are repetitively obtained because these ganglia, supercharged as it were from above, resist the blockade which, in other animals, supervenes after repeated TEAC dosage As con

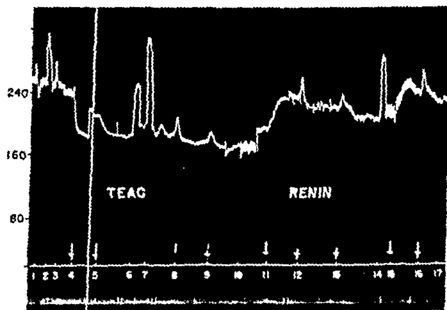


FIG 2 Responses of a renal hypertensive dog (Both kidneys wrapped in cellophane Unanesthetized blood pressure 252 mm Hg)

(1) Adrenaline (2) Noradrenaline (3) Barium chloride (4–5) TEAC 5 mg/kg (6) Adrenaline (7) Noradrenaline (8–9) TEAC (10) Barium chloride (11) Renin (12–13) TEAC (14) Adrenaline (15) Renin (16) TEAC (17) Barium chloride (no 968)

cerns clinical hypertension the point of interest lies in the fact that a minority of patients show repetitive deep depressor responses to TEAC, whereas the majority do not, the response pattern of those who do this corresponds to that of dogs with sino aortic hypertension and suggests that hypertension in these patients is also primarily neurogenic It is hoped that

depress amine oxidase activity, although this would not seem to apply to histamine barium or sodium nitroprusside The hypothesis was tested by measuring responsiveness to noradrenaline serotonin and angiotonin before and after administration of drugs which *in vivo* or *in vitro* were known to

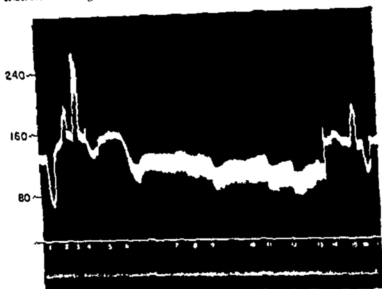


FIG 4. Refractoriness in a normal dog resulting from inhalation of 30 per cent carbon dioxide

(1) Nitroprusside (2) Adrenaline (3) Noradrenaline (4) Sodium azide (5) Barium chloride (6) 30 per cent carbon dioxide (7) Adrenaline (8) Noradrenaline (9) Nitroprusside (10) Barium chloride (11) Sodium azide (12) Nitroprusside (13) pH 6 93 Air given. (14) Adrenaline thirty five minutes later (15) Noradrenaline (16) Sodium azide Exclusion of both carotid sinus nerves altered this pattern insignificantly (From Page and Olmsted, 1951 Circulation 3 801)

inhibit amine oxidase Among these ephedrine gave slight augmentation of response to noradrenaline but disappointingly amphetamine had either no effect or even impaired the responses and a third agent Marsalid (1 isonicotinyl 2 isopropyl hydrazide phosphate) clearly decreased them

Curiously, the effect on angiotonin responsiveness is not as great when hexamethonium is substituted for TEAC

Thus, under specific situations, discrete neural ablations result in highly characteristic changes in reactivity But the complexity of the problem and the sensitivity of the mechanisms involved is illustrated by the fact that enhancement of angiotonin response by buffer nerve section is only observed when TEAC is given by a definite schedule of repeated doses, when either TEAC or hexamethonium is given by slow infusion, the augmentation which results resembles that seen after cord section regardless of the state of the buffer nerves Thus it seems likely that we deal in these situations with the interaction of selective neural mechanisms of vascular regulation, their existence has only been visualized and their nature is not understood

Another aspect of the neural control of reactivity is demonstrated by causing anæsthetized, curarized dogs to inhale 15–30 per cent CO (Page and Olmsted, 1951) They respond by a moderate decrease of arterial pressure, at this point the ability to respond to adrenaline or noradrenaline is either greatly impaired or absent responsiveness can be restored or the onset of refractoriness prevented, by ganglionic blockade or by ablation of the paravertebral sympathetic ganglia

These examples of alterated responsiveness show that neural regulation of circulatory function is exercised in large part by mechanisms which control and inhibit and do not exclusively work, as we have been accustomed to think, by means of excitation The limited data available prevent any very specific conclusion, they do suggest that those interested in circulatory controls should concern themselves with the inhibitory as well as the excitatory aspects of neural regulation indeed by analogy it seems likely that circulatory neural controls like other neural functions act by a system of equilibrating ' feed backs'

One of the more attractive explanations of the sensitizing action of the blocking drugs has been that they somehow

depress amine oxidase activity although this would not seem to apply to histamine barium or sodium nitroprusside The hypothesis was tested by measuring responsiveness to noradrenaline serotonin and angiotonin before and after administration of drugs which *in vivo* or *in vitro* were known to

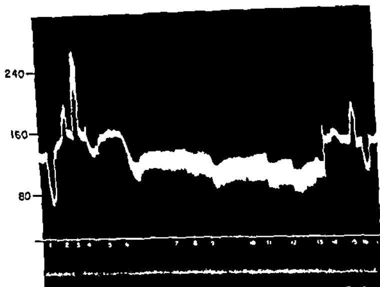


FIG 4 Refractoriness in a normal dog resulting from inhalation of 30 per cent carbon dioxide

(1) Nitroprusside (2) Adrenaline (3) Noradrenaline (4) Sodium azide (5) Barium chloride (6) 30 per cent carbon dioxide (7) Adrenaline (8) Noradrenaline (9) Nitroprusside (10) Barium chloride. (11) Sodium azide (12) Nitroprusside (13) pH 6 93 Air given. (14) Adrenaline thirty five minutes later (15) Noradrenaline (16) Sodium azide Exclusion of both carotid sinus nerves altered this pattern insignificantly (From Page and Olmsted 1951 *Circulation* 3 801)

inhibit amine oxidase Among these, ephedrine gave slight augmentation of response to noradrenaline but disappointingly amphetamine had either no effect or even impaired the responses and a third agent Marsalid (1 isonicotinyl 2 isopropyl hydrazide phosphate) clearly decreased them

Thus, inhibition of amine oxidase activity would not seem to be the reason for the post TEAC augmentation

The importance of autonomic nerve function in vascular response to a vasoactive agent, is exemplified dramatically

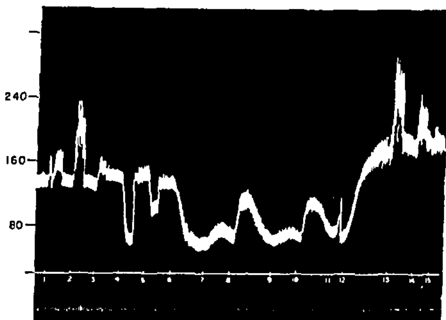


FIG 5 The effect of 30 per cent carbon dioxide on vascular reactivity in a sympathectomized dog (No 1828)

(1) Adrenaline (2) Noradrenaline (3) Barium chloride (4) Nitroprusside (5) Sodium azide (6) 30 per cent carbon dioxide (7) Adrenaline (8) Noradrenaline (9) Adrenaline (10) Noradrenaline (11) pH 6 80 (12) Air (13) Adrenaline (14) Noradrenaline (15) Adrenaline This shows the relative maintenance of responsiveness even after a very severe fall in arterial pressure and the period of supersensitivity when carbon dioxide is withdrawn quickly followed again by normal or reduced reactivity (From Page and Olmsted 1951 *Circulation* 3 801)

in the case of serotonin This drug has usually a biphasic depressor pressor response in the dog in the cat the response *is purely depressor as it is also in the dog with neurogenic* (sino aortic) hypertension Blockade of autonomic ganglia with TEAC or hexamethonium causes the drug to exert a

purely pressor action (Page and McCubbin 1953) Thus response to serotonin is an index of vasoconstriction resulting from nervous activity

The drugs which block the actions of the sympathetic nervous system more peripherally result in still another pattern of reactivity Regitine Priscoline and the Benzo dioxanes are presumed to interfere with the humoral mediators adrenaline and noradrenaline at the myoneural junction Such blockade again exemplifies the specificity and varied nature of changes in reactivity These agents reverse the response to adrenaline and inhibit the pressor response to noradrenaline but do not affect pressor responsive ness to angiotonin and Pitressin Serotonin is affected irregularly by adrenergic block Under certain conditions its action may be transiently reversed from pressor to depressor

To sum up, a variety of neural activities influence not only the level of arterial pressure but also and with some speci ficity responsiveness to vasoactive agents (Page and Taylor 1950) These effects may depend on one and the same action variou ly exerted The data at hand emphasize the impor tance of considering not only the amount and kind of vaso-excitor which may be present but also the amount and kind of response which it will excite the concept is *proposed that* the inhibitory as well as the excitatory properties of central nervous function must be considered in assessing the nature of drug action In a world of cats and neurogenic hyperten sive dogs serotonin would be classed as vasodepressor in the world of reality we find that its properties depend on the responsiveness of the *recipient*

Humoral Controls

The relationship between the presence of humoral sub stances the calibre of blood vessels and their reactivity has been under study for many years The brilliant studies of von Euler of several English physiologists and Peter Holtz adumbrated by Walter Cannon have uncovered much of the mystery of adrenaline and noradrenaline Nature s experi

ment in this field, the phæochromocytoma, has demonstrated to the clinician the havoc that a disequilibrated humoral system can produce In spite of the relative simplicity of the chemical structure of the adrenergic agents progress in this field has taken a good many years The structural complexity and small amounts of other potentially significant humoral agents probably account for the fact that we know so little about them And, fortunately or not, Nature does not co operate by establishing tumours which will secrete angiotonin, serotonin, cerebrotonin, either alone or in combination

Renin, Renin Substrate, Angiotonin (*Renin Hypertensinogen, Hypertensin*) The study of the renal pressor system began when Tigerstedt and Bergmann showed that extracts of kidney were pressor when injected intravenously into dogs The substance responsible for this effect was termed renin and considered to be a pressor substance with direct action This concept was dispelled when with Helmer, we found that initial purification of renin yielded a protein mixture which did not act directly on the blood vessels of isolated organs perfused with Ringer's solution (Page and Corcoran 1948) These mixtures were then found to become vasoconstrictor when perfused in the presence of plasma The substance in plasma which made the inactive renin active was tentatively called, "renin activator' (Kohlstaedt *et al*, 1940 Page 1940) until further studies showed that what had occurred was an enzymatic interaction of renin with a protein substrate and the formation of an ultrafiltrable pressor substance which was directly vasoconstrictor The substrate was termed renin substrate and the pressor product of the reaction angiotonin Eduardo Braun Menendez accomplished a similar demonstration at the same time, and he and his associates termed the substrate hypertensinogen and the product of the reaction hypertensin (Braun Menendez *et al* 1946)

At the time we both probably had the notion that scientific nomenclature was an ordered system and that each in his

own way had fulfilled the rules and regulations while the other had flouted them We have both learned that the rules of nomenclature seem to be mostly self made and although we now laugh at the tangle we created we regret the difficulties experienced by medical students in assimilating this terminology Unfortunately the trivial names will persist for while it would be desirable to use chemical names it is unlikely that anyone ever will these materials are too complex Even as concerns simpler compounds while 5 hydroxytryptamine may replace serotonin and enteramine neither adrenaline nor noradrenaline are referred to by their more informative structural names and Regitine' is more convenient than 2(N p tolyl N m hydroxyphenylamino methyl) imidazoline

The clue to the nature of the renal pressor system was the demonstration of renin substrate However this material is not yet available in pure form Some years ago with Plentl and Davis we found (Plentl *et al* 1943) that most of the renin substrate of plasma occurred in the alpha 2 globulin Green in our laboratory has since prepared a concentrate of this material by fractionation of plasma with ammonium sulphate at various pH s One gram of this concentrate yields on incubation with renin 20 to 30 mg of a peptide fraction which corresponds in activity to 6 mg of the purest angiotonin yet prepared This small yield testifies to the probability that the substrate content of the concentrate must still be relatively small in relation to the other proteins it contains In brief all we know is that renin substrate is probably an alpha 2 globulin that it is formed in the liver (Page *et al* 1941) and as Dr Helmer will tell us that its formation is influenced by the adrenal corticosteroids

A significant advance has been made in the preparation of renin in that Haas Lamfrom and Goldblatt (1953) have obtained a material of very great potency Unfortunately even after 34 000 fold purification electrophoretic analysis reveals two components and the estimated purity of the material is about 65 per cent This material however is

much more homogeneous than any which has hithert available Thus, the studies done to date on renin and substrate, on their interaction and concentrations in bloo tissues, have all ultimately to be re examined by n enzymatic methods as purer reactants come into our l Many early claims and counter claims can be put asid judgement suspended until confirmation by exact me possible

Thus, with an impure substrate and an impure enzym is no wonder that the isolation of the complex product of interaction has not gone on apace Angiotonin has been n in my thoughts for the past fourteen years as, under ano name but just as elusive, it has been in those of Dr Br Menendez Our group including my former associa Helmer and Plentl made great advances in its purificati only to be outstripped by Edman in Sweden, but Edn seems to have lost interest in the problem Our pres colleague Bumpus, has recently prepared an angiotonin th is as nearly as the comparison can be made, more than tw as active per milligram nitrogen as Edman's best

The fascination of angiotonin lies partly in its ability t mimic most of the hæmodynamic changes of essential hype tension Unfortunately pending better methods the mos that can be said is that it seems to be present in increase concentrations in the blood of some renal hypertensive animal and of some patients with advanced hypertensive disease However in this connection one should keep in mind the point made above that under appropriate conditions of altered neural function responsiveness to angiotonin is greatly increased Consequently it is possible to envisage a situation in which normal or presently unmeasurable con centrations of this agent might effect a large and persistent increase in arterial pressure And once the new equilibrium of arterial pressure had become established the enhanced sensitivity to angiotonin which set the process in motion might very well disappear Admittedly the situation I envisage here is complex and hypothetical I point it out



partly because this field is one in which oversimplification is both easy and disappointing

Lastly in this connection some mention should be made of the demonstration by Masson Corcoran and Page (1951 1952) that administration of renin to rats pre treated with salt and deoxycorticosterone cortisone or hydrocortisone results in diffuse vascular lesions which do not appear in non sensitized rats In particular the syndrome which occurs in rats pre treated with deoxycorticosterone and salt has a superficial resemblance to eclamptogenic toxæmia of pregnancy and in view of the known associations of this disease with sodium retention on the one hand and renal damage on the other may have a definite clinical implication The mechanisms of interaction of renin angiotonin and salt in the establishment of these vascular lesions is unknown The lesions are impressive (Fig 6)

Cerebrotonin About eighteen years ago I found a strong pressor substance in the fluid removed from the cerebral ventricles of patients and animals (Page 1935) It was not regularly present but its demonstration led us to search further for vasoactive agents of central nervous origin After making a number of necessary changes in the usual techniques Taylor and I finally developed a procedure for perfusion of the isolated dog s head that has implemented this search (Taylor *et al* 1951 Taylor and Page 1952)

It seemed likely that the demonstration of possible vaso active properties in the central nervous system might best be accomplished under conditions of strong central stimula tion Electrical stimulation of the central end of the cut vagus nerve is known in normal anæsthetized dogs to elicit a large pressor response This pressor response is generally ascribed to reflex sympathetic stimulation with adrenal medullary discharge However it now appears that several factors may participate in this response and so many have now been demonstrated that it becomes hard to isolate and quantitate each separately

Barreda Jimenez Diaz and Molina (1947 1948) attribute

at least part of this response to neurogenic activation of the extrarenal "renin" they find in vessel walls, more recently, they attribute the response to liberation of noradrenaline, also from vessel walls the distinction, if such is intended, between this mechanism and the well established fact that noradrenaline is released at sympathetic nerve endings, is not yet clear in my mind We shall look forward to hearing their current views and information on this point

Taylor and I have found that, under certain conditions, stimulation of the central end of the cut vagus results in pressor responses in the body after cord section has eliminated the possibility of sympathetic reflex discharge We attribute this aspect of the pressor response to the liberation by the brain into the blood of a pressor substance which, pending identification, can be called cerebrotonin I state the name reluctantly, since what we have to deal with is a phenomenon and not a substance, still, to give it a name is both fashionable and convenient

The cerebrotonin response is not attributable to release of adrenaline or noradrenaline, since drugs which inhibit or reverse the pressor activities of these amines potentiate the response to vagus stimulation Nor does it seem attributable to mechanical expression of blood from the constricted vessels of the brain into the circulation of the body, since adrenergic blockade prevents vasoconstriction in response to sympathetic stimulation Apresoline (1 hydrazinophthalazine), which has little effect on responses to noradrenaline, adrenaline, Pitressin, renin and angiotonin, prevents or reverses the cerebrotonin response a preparation of mixed veratrum alkaloids (Veriloid) acts similarly while protoveratrine is inactive in this respect Neither hypophysectomy nor the establishment of renin tachyphylaxis prevents the response However, the participation of Pitressin in the response has not been wholly excluded, since this material occurs in the diencephalon as well as the posterior hypophysis

Little more is known concerning this phenomenon Our present and necessarily tentative conclusions are that cere

brotonin is a pressor substance of midbrain origin which differs from other known pressor substances with the possible exception of Pitressin No data are available as to its nature or physiological function nor have we yet identified it with the substance found a number of years ago in ventricular fluid Clearly this is a fertile area for further investigation we hope that it will soon be possible through the joint efforts of our group and our Spanish colleagues to delineate clearly the several mechanisms that raise arterial pressure when the brain is stimulated by passing an electric current through the vagus nerves

Serotonin This is the last of the 'tonins which I will discuss I have been plagued for years by its occurrence in drawn blood in which a vasoconstrictor would unaccountably appear and disappear it constantly confused the search for circulating vasoactive agents while it interfered also with nicely contrived attempts at organ perfusion

Over the course of several years we had developed the technique of perfusion of the rabbit ear to a point where it could be used in semi quantitative assay Consequently when Green and Rapport joined our group the rabbit ear method was used in the resolution of the problem The serum vasoconstrictor was isolated in 1946 as the creatinine sulphate complex and its structure partially determined in 1947 (Rapport Green and Page 1948*a* *b*) Two years later the complete structure was reported (Rapport 1949) as 5 hydroxytryptamine this material was synthesized by Hamlin and Fisher (1951) and others soon after The pharmacological activities of the natural and synthetic serotonin we found to be identical

The late Dr G Reid of Melbourne was certainly among the first to add substantially to the pharmacological knowledge of serum vasoconstrictor he gained a considerable insight into it in spite of the fact that he was forced to do most of his work with impure preparations

A quite different but very productive approach was that of Erspamer who a number of years ago prepared acetone

extracts of tissues and found in the gastrointestinal tract and spleen of mammals and in extracts of octopus a weakly vasoactive material which he called "enteramine", and provisionally identified as a polyphenolic amine In view of his widely different approach it is not surprising that none of us suspected that serotonin and enteramine were the same substance under different names

Now that identity has been established, Erspamer's studies (Erspamer and Boretti, 1951, Erspamer and Ghiretti, 1951, Erspamer and Asero 1952) and ours (Twarog and Page, 1953) show that serotonin is widely distributed It occurs in venom, clam muscle salivary gland of octopus amphibian skin mammalian intestinal tract and spleen Recently, we have found relatively high concentrations in dog, cat and rabbit brain and concentrations identifiable by clam heart assay and paper chromatography in normal human and dog urine The amount normally present in unclotted blood must be very small, assays of human blood, like those on extracts of mammalian kidney and heart are complicated by the presence of a substance which impairs the responsiveness of the clam heart preparations

The fact that serotonin forms complexes with creatinine and that it is widely distributed in muscle and nerve tissue suggests that it may be an important link between muscle and nerve function Dr Twarog found that it occurs in the byssus retractor of *Mytilus edulus* and believed that it might be the physiological relaxor of this smooth muscle from contraction induced by acetylcholine or electrical stimulation A mammalian counterpart of this is found in dogs with experimental neurogenic hypertension, in these vascular smooth muscle brought into contraction by vasomotor impulses is relaxed by serotonin injections

However its part in mammalian physiology is still undecided and its pharmacology complex As McCubbin and I found (Page and McCubbin, 1953), the degree of pre existing vasomotor tone largely determines whether its major effect is pressor or depressor, elimination of neurogenic

tone results in pure pressor responses in cats rabbits and dogs

Other cardiovascular properties of this agent are that it is a direct vasoconstrictor that it elicits a von Bezold like

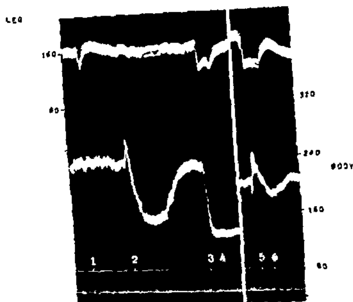


FIG 7 Comparison of hypotensive action of serotonin and TEAC in a neurogenic hypertensive dog with hind leg perfused with blood of a donor animal *Top tracing* is perfusion in leg measured by mercury manometer inserted into circuit between pump and leg Bottom tracing is arterial pressure of body Pressure of donor animal not registered

(1) Serotonin 0 003 mg directly into leg ( ) Serotonin 0 06 mg into body (3) TEAC ~ mg/kg into body (4) Serotonin 0 003 mg into leg (5) Section sciatic and *femoral* nerves (6) Serotonin 0 003 mg into leg

action and transiently blocks impulse transmission through sympathetic ganglia (Page and McCubbin 1953) These various activities contribute to the complexity of its effect on arterial pressure Typically in normal dogs it elicits a rapid depressor followed by a sharp pressor response (Fig 7)

the initial depressor phase is attributable to the von Bezold reflex and ganglion blockade and the pressor phase to its vasoconstrictor action, the subsequent, more prolonged, depressor phase of the response is attributable to its peripheral action in reducing neurogenic tone and relaxing smooth muscle (Page, 1952) The response in normal cats is purely depressor, and differs in this respect from that usually observed in dogs and man, while it resembles that seen in neurogenic hypertensive dogs (Fig 8) These differences prompted

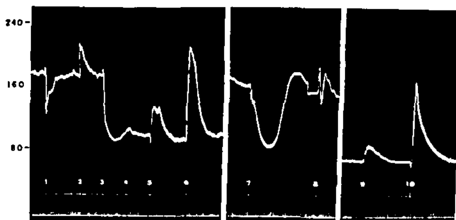


FIG 8 Normal cat showing depressor response to serotonin with reversal to pressor after TEAC and after pithing

(1) Serotonin 0 03 mg (2) Noradrenaline 2 5 µg (3–4) TEAC 5 mg /kg (5) Serotonin 0 03 mg (6) Noradrenaline 2 5 µg recovery from TEAC (7) Serotonin 0 03 mg (8) Noradrenaline 2 5 µg pithed (9) Angiotonin (10) Serotonin 0 03 mg

examination of the responses of a number of hypertensive patients to the drug the responses vary widely from depressor to pressor, the more common responses resemble those usually seen in normal dogs while some patients show the depressor responses which characterize increased neurogenic tone it is hoped that further clinical correlation of these responses may uncover some of the mechanisms of human hypertension

Serotonin has also respiratory effects (Douglas and Toh, 1953 Comroe, 1952) which differ between species in cats it

causes apnœa and in dogs hyperpnœa as it does also in man (Page 1952) These do not contribute importantly to its effect on arterial pressure

While it has been suggested that serotonin is antidiuretic and even an antidiuretic hormone Corcoran del Greco and I have been unable to demonstrate that it has significant antidiuretic or renal vasoconstrictor properties in normal dogs given serotonin by intravenous infusion Under these conditions serotonin is depressor and the decreases observed in effective renal plasma flow filtration rate or urine flow are attributable to hypotension as such Mild depressor responses to serotonin are commonly associated with moderate transient renal vasodilatation rather than vasoconstriction Of course injection of a large dose of serotonin especially when given subcutaneously might well initiate a chain of events which would result in release of antidiuretic hormone such responses however, can have little to do with its place in mammalian physiology Perhaps still its most obvious role is that of a local vasoconstrictor at the site of blood coagulation

Erspamer and Asero s observation (1952) that the smooth muscle stimulating properties of acetone extracts of intestine are due chiefly to serotonin has been confirmed by Dalgliesh Toh and Work (1953) However this association is complicated by the smooth muscle stimulant found by von Euler and Gaddum (1931) in alcohol extracts of intestine and brain which they called substance P It now seems that serotonin and substance P are not identical (Pernow 1953) and that substance P is probably a polypeptide Details of this discussion I must leave to others

We prepared some years ago a protein extract of lung which destroys serotonin at rates corresponding to those of first order reactions (Rapport Green and Page 1948c) Tissue extracts which destroy serotonin have also been prepared by Erspamer It remains to be demonstrated that the enzymatic degradation is specific that it may be due to amine oxidase has been suggested at least it is rapidly oxidized

by this enzyme (Blaschko, 1952) By clam heart assay, we found that it is rapidly inactivated when infused into animals, even during the infusion, little can be demonstrated in distant blood, variable proportions (3 to 20 per cent) of injected serotonin appear in urine

Udenfriend, Clark and Titus (1953) have found hydroxy tryptophan in normal blood and have demonstrated an enzyme in kidneys which specifically decarboxylates this amino acid to yield serotonin Its association with blood clotting and blood platelets is not apparent from this scheme of its formation As to its degradation product, the most relevant analogy is the demonstration (Ewins and Laidlaw, 1913) that tryptamine is degraded by the liver to indoleacetic acid

V D M and V E M While I have no first hand experience with this problem, I have followed it from its inception with interest Chambers and Zweifach developed a method which measured the reactivity of the small vessels of the rat s mesoappendix to adrenaline Shorr and Zweifach later applied the method to an extraordinary variety of experimental situations and arrived at an elaborate systemization of the vascular reactions taking place You are all familiar with the attractive formulation and it has received wide acceptance in both the clinical and experimental literature

It is no secret that I have been somewhat dismayed by the uncritical acceptance of these interesting theories To me the problem rested on whether (1) the rat mesoappendix test was sufficiently quantitative to allow the confident figures which were given, (2) whether the changes in reactivity were specific to one substance such as V D M or to a variety of substances, (3) whether it is justified to reason that changes in reactivity in one vascular area may be translated into terms of arterial pressure in the entire circulation With the finding that V D M and ferritin were identical it was to be anticipated that the administration of this substance would lead to profound vascular changes As you know this has not proved to be true

Study of the arteriolar and capillary beds is most desirable and I am sure a method will be found to make it objectively The current subjective definition of end points by observation under the microscope while dripping adrenaline on the outside of the vessel leaves much to be desired There are so many factors which can influence reactivity, that it would be surprising if only a specific V D M or V E M were involved It remains to be shown that the test can be used as a quantitative measure of the relative amounts of these two hypothetical substances and that this can form the basis for elucidation of the mechanisms in shock and hypertension To put it bluntly I believe theory has well outrun experiment the pyramid is resting on its point rather than its base the point being the inadequately explored and documented rat mesoappendix test The problem needs careful and sympathetic re evaluation

Reactivity and Organ Systems of Unknown Mechanism

Apart from the neural controls of reactivity there remain other organs which have both broad and selective effects on this function The first of these are the kidneys

These have for years been known to inhibit responses to renin and angiotonin McCubbin and I (1954) have found that the augmented responses to renin and angiotonin which occur after nephrectomy are not related to excretory renal function but to the presence of intact kidney tissue This is an interesting homeostasis in that the kidneys seem to regulate responses to the very substances they form In contrast to so many other aspects of the study of hypertension neither the nervous system nor the sodium ion participate in this aspect of renal function

The liver seems to have a much less selective influence (Page 1950) Reactivity to most vasoactive substances is impaired or lost soon after hepatectomy Loss of reactivity is usually associated with a decrease in arterial pressure Since many vasoactive agents are presumably conjugated or inactivated in the liver it might have been supposed that

hepatectomy would increase their effectiveness The fact that the reverse is true suggests that the liver adds something to the blood which is essential to normal muscular contraction

The thyroid gland seems also to be concerned in reactivity In dogs at least, severe chronic hypothyroidism depresses vascular reactivity (Page and McCubbin, 1952*b*) the mechanism of this response is the more obscure in that administration of desiccated thyroid further impairs and does not restore responsiveness to vasoactive drugs

Neither the pituitary nor the gonads seem to have distinct influences on reactivity Adrenalectomy without replacement therapy results in a transient augmentation followed by a progressive decrease in reactivity to renin, while angiotonin responses persist although they may be impaired this difference in response could be explained as a result of deficient renin substrate Reactivity to other vasoactive agents is not much impaired by adrenal failure Except as the adrenals may participate in formation of renin substrate, their influence on reactivity in general is relatively non specific

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DISCUSSION

PICKERING I should like to start this discussion by asking a question about the reactivity which was your first point Have you in fact been able to satisfy yourself as to any alteration in reactivity in human hypertensives?

PAGE. Yes In the slide that I showed (Fig 3) roughly 10-15 per cent

of the hypertensive population responds by a repetitive fall in blood *pressure to tetraethylammonium chloride (TEAC) That is what we* call the chronic response pattern In other words instead of show*ing an initial depression followed by a reversal to a rise in blood* pressure, these neurogenic hypertensive dogs always show a fall in blood pressure *A certain percentage of hypertensive patients show* exactly the same thing It will be interesting if we can show that *those people respond to total sympathectomy I would like to point* out that some of our associates here have nearly ruined our chance of this—*we cannot persuade people to be sympathectomized any more* simply because they want to be treated with hexamethonium instead It makes it very difficult from the scientific point of view Does that answer your question?

PICKERING Partly You haven't any evidence that these patients behave in other ways as though their hypertension were neurogenic?

PAGE No Curiously enough they are not the group we have described as the hypertensive diencephalic syndrome This syndrome along with the one we call ancestral has only circumstantial evidence in favour of its being of neural origin Examination of the patients showing this chronic response pattern with TEAC does not suggest any especial hyperactivity of the nervous system in other words they do not show the emotional disturbances and exaggerated activity of the autonomic system that we formerly regarded as being characteristic of the neurogenic hypertensive

HELMER Could you use the Amytal sedation test?

PAGE Not necessarily I doubt if there is anything specific about the Amytal sedation test In fact TEAC 5 hydroxytryptamine and a few others like alstonine among all the drugs that we have tried on patients *are the only ones that show a differential response from normal* Veratramine is useful in dogs with experimental hypertension but it *has some dangers connected with its use such as producing convulsions*

PICKERING If you were trying the reactivity to a specific substance and *were testing it by measuring the blood pressure would you take* the absolute response of the blood pressure or would you take the *response of the blood pressure as a percentage of its initial value?*

PAGE I would prefer the absolute response if I had to choose actually it is the pattern of response which chiefly interests us It is a fact that the height of the blood pressure need not determine the degree of response In renal hypertensives for example most depressor and pressor drugs give the same absolute fall or rise seen in a normotensive Contrasting with these the neurogenic hypertensive gives a much greater fall with some depressor drugs but not with others It is this differential that we are using to try to classify our hypertensive patients We have I think clear evidence that patients who appear much the same on clinical examination are quite different in the patterns of their response to different drugs I believe this is the reason why the response to the various therapeutic agents is so varied in different patients In short we work from the patterns *determined in animals with known* forms of hypertension to the patients in whom it is unknown

PLATT What about renal hypertensive human beings—cases that clinicians have assumed were cases of renal hypertension cases of *chronic* renal disease?

PAGE For instance chronic Bright s disease?

PLATT Yes

PAGE They show a normal pattern but I hope sooner or later we shall be able to demonstrate some abnormality I am sorry to say that the response to single injections of angiotonin seems to be quite normal

PLATT You have tried them with repeated injections of TEAC do they all show a normal pattern?

PAGE Yes so far as we have gone I should add that the pattern for instance with TEAC can be altered by the salt content of the diet consequently it is necessary to be aware of the treatments the patient is receiving

PATON There is a point in the pharmacology of TEAC I should like to make You did not mention the way that with biggish doses in animals it can produce a secretion of adrenaline and noradrenaline I wonder therefore whether the distinction between your groups may be nothing particularly neurogenic but may lie in the ability of the animal to build up a secretion of adrenaline or noradrenaline which would then conceal ganglion block or something of that sort Have you controlled that?

PAGE The problem you bring up is a most interesting one Our early work with Dr Taylor suggested that large doses of TEAC caused liberation of noradrenaline and adrenaline I suppose you have in mind that the transient ganglion blockade by those substances might be at the basis of the *response* pattern in neurogenic hypertension I don t know whether it is true or not but it seems to me a very discerning suggestion Unfortunately if we block the action of noradrenaline with Regitine the hypertension is difficult to maintain The best I can do for you is to say that when I elicit tachyphylaxis with amphetamine then the chronic response pattern to TEAC disappears Do you suppose amphetamine is doing what we are suggesting for endogenous noradrenaline? *Incidentally* hexamethonium exhibits much the same pattern as TEAC

PATON You really want a *transient* ganglion blocking agent free of that adrenaline producing activity

PLATT Might I suggest that dihydro-ergotamine might answer part of the problem?

PAGE It should but unfortunately it does not DHO-180 often does give a much *greater response* in the neurogenic hypertensive than in the normal

BRAUN MENENDEZ I have repeated Dr Page s experiments in the rat and it behaves differently from the dog and the cat Repeated injections of TEAC in hypertensive or normal rats always give depression of blood pressure As to the effect of serotonin— you cannot tell what will happen It may give hypertension and then in the same animal a marked hypotension and so on

PAGE I certainly agree with Dr Braun Menendez that serotonin is

unpredictable But in view of what I have shown I would say that probably the unpredictability depends on its ability to block peripheral neurogenic tone The unpredictability of serotonin led us to suggest amphibaric as a term descriptive of its action on the circulation

BRAUN MENENDEZ I mean repeated injection of serotonin in the same animal You give one two injections with a pressor effect then the third injection gives hypotension then hypertension again and so on It may depend on the level of blood pressure at the moment of injection I have done the experiment in anaesthetized and in non anaesthetized animals

PAGE I think that is because the vasomotor tone changes We would say that in the neurogenic hypertensive animal in cats and even in dogs under some conditions the tone is high and that the tone is one of the chief determinants in the response elicited In the neurogenic hypertensive serotonin gives a fall—when the tone is high it gives a fall If the tone is abolished by section of the cord by pithing or by TEAC or hexamethonium then it gives a rise

BRAUN MENENDEZ I have not checked that

PATON You said in your paper that hepatectomy produced a decrease in responsiveness You did not say how long it took for that to develop although I believe you have published this

PAGE It takes about 3–8 hours after the hepatectomy and I suspect that it depends a great deal on the skill with which the hepatectomy is done, because we found that as our skill improved the refractoriness developed more slowly

PATON Starting at about 3 hours and going on progressively?

PAGE Yes although the onset and rate of development vary greatly

BING Did you try partial hepatectomy?

PAGE Partial hepatectomy in our hands does not do anything We tried a variety of partial surgical hepatectomies and also occlusion of the hepatic artery after an Eck fistula which we found very difficult to perform so as to produce hepatic ischæmia When we did manage it it did not do anything that seemed specific but the animals were of course very sick which might have obscured a more definite result

HELLER Did you try injury of the liver with chemical substances?

PAGE Yes carbon tetrachloride poisoning of a rather extreme degree does produce it but it is not very satisfactory The lesion is so variable you get some effect but it is not a very convincing experiment We used it in our published report merely as supporting evidence for total surgical hepatectomy

PEART Dr Page is the effect of the hepatectomy due completely to reduction of portal blood flow? Does removal of the viscera or the spleen supply the effects?

PAGE No it does not

VON EULER I was wondering whether large doses of antisympathetic substances would inhibit that rise in blood pressure I ask because Dr Solero in Rio de Janeiro using one of Dr Bovet s very effective sympatholytics found that on stimulation of the central end of the vagus he could still get a rise in blood pressure even when injected

noradrenaline had no effect Using bigger doses he found that both the effect of central vagus stimulation and carotid occlusion disappeared simultaneously

PAGE. I think that is a very relevant criticism. The problem of blockade is a very difficult one To elicit it very large amounts are required in some cases practically intoxicating the animal Further if the animals are not in good condition you do not get a good response We have stressed the point that it is not always easily reproducible Augmentation with TEAC and the blockade with Apresoline—almost a specific blockade—strongly suggest a pressor substance with unusual qualities I think we would all agree that the experiments as they are done are pretty traumatizing If we could work under conditions in which there was less trauma to the brain and to the whole animal maybe we would get more easily reproducible results

PICKERING. Might I ask another question about that last provoking slide (Fig 6) which you showed us? What happens to the arterial pressure of those rats? Is the material you showed us the same as the fibrinoid that you get in the fibrinoid lesions of malignant hypertension? And is it confined to blood vessels or does it occur elsewhere?

PAGE. The lesions are almost identical with those of malignant hypertension It is interesting because Masson Corcoran and I have recently shown that such different adrenal steroids as DCA and cortisone or hydrocortisone sensitize to the destructive action of renin on blood vessels The material in the vessels looks like fibrinoid and has the staining qualities of it and is largely confined to blood vessels But when necrosis of the blood vessel begins it spreads out for instance in lesions of the myocardium. The fibrinoid material seems to be of blood origin much as though from plasma that had been extruded The lesions are very dramatic widespread and associated with a massive oedema when DCA is used as sensitizing agent But the same type of vascular lesion occurs after hydrocortisone without development of oedema The blood pressures of the animals have not been entirely satisfactorily because the *animal's tail* is also oedematous making it very difficult to measure blood pressure except under anaesthesia Hypertension of some degree is doubtless present in many of the animals The condition has many of the qualities of toxaemia of pregnancy even to the convulsions To me these experiments demonstrate extraordinary properties of renin not suspected before Even if its pressor action proves not to be the cause of the elevation of blood pressure in some forms of hypertension—which I am not prepared to grant—its vasculotoxic effects are not to be ignored in the aetiology of malignant hypertension

LEDINGHAM Is it associated with severe proteinuria beforehand?

PAGE Yes they have severe proteinuria

LEDINGHAM Before the lesions or after the lesions?

PAGE. That is a little hard to say they develop it along with the lesions which occur very rapidly within twenty four hours It is a very acute process

GOVAERTS May I ask how much deoxycorticosterone acetate and how much renin you have to give to those rats?

PAGE Two 35 mg pellets of DCA were implanted About 0 75 ml of renin was given daily in three subcutaneous injections One tenth ml of this renin gave a rise of 35 mm Hg in a dog under pentobarbital anaesthesia

GOVAERTS That is a large dose for a rat This is the trouble with many experiments on the rat that when you translate the dosage to what we are accustomed to see in humans, it corresponds to terrific doses

PAGE I think we all recognize and I tried to point out in my talk that these things sometimes smack of being scientific tours de force and yet in most cases we have no choice but to do acute experiments to point the way to the chronic ones more nearly mimicking Nature s own You can argue that you get the lesions in human beings simply because it is a process that is going on and on they are getting small amounts of cortisone or hydrocortisone and small amounts of DCA and small amounts of renin day in and day out It doesn t worry me too much on that count

HEYMANS It seems that it is quite difficult to compare the reactions in blood pressure of the normal dog and the dog deprived of his sino aortic presso receptor mechanisms

In the normal dog, injections of hyper or hypotensive drugs induce also compensatory reactions to limit the rise or fall of arterial pressure These compensatory reactions are absent in the dog deprived of his sino aortic innervation and the blood pressure responses are quite different The starting level of the arterial pressure is also an important factor in the responsiveness and the pattern of reactivity to a given compound The blood pressure level is higher in the debuffered animal than in the normal one Could not these factors interfere in your experimental conditions?

PAGE I am afraid Prof Heymans that I did not make myself quite clear I think your point is very well taken I have tried to indicate that our evidence shows a basic difference in the pattern of response to such drugs as TEAC and to serotonin When neurogenic hypertension is elicited TEAC doesn t just cause a little more of the same action as it does in normal dogs The whole type of response changes The same is true of serotonin and other drugs But interestingly enough the response to many other vasoactive drugs is little changed For example noradrenaline and angiotonin behave pretty much as in normal dogs Even some depressor drugs do not lower arterial pressure to a much greater degree than in normal animals although the starting point may be 290 mm Hg instead of 120 There is much more specificity of action than at least McCubbin and I would have suspected It is this specificity which holds for hypertensive patients as well as the hypertensive dogs we hope will help us classify patients objectively

SOME NEW ASPECTS OF REFLEX BLOOD PRESSURE REGULATION AND HYPERTENSION

C HEYMANS

It is known that the aortic and carotid sinus nerves not only provide the means of physiological regulation of blood pressure, but are also the reflex buffer or moderator nerves of the systemic arterial pressure This reflex regulation of blood pressure occurs through the action of the arterial pressure itself on receptors located in the vascular wall of the sino aortic areas

Experiments by Koch (1931) Heymans and co workers (1933) and Hauss and associates (1949) have shown that arterial pressure does not act directly on the sino aortic receptors but indirectly by stretching the wall of the arteries where the sino aortic receptors are located

These experimental observations suggested that the state of contraction tone tension and distensibility of the arterial wall of the sino-aortic areas could play a role in the mechanisms of reflex regulation and homeostasis of arterial pressure

This suggestion has been investigated in a series of experiments in order to examine the influence on arterial pressure and on the reflex regulation of blood pressure of changes in tension resistance to stretch and distensibility of the sino aortic vascular wall For this purpose drugs known or supposed to contract or to relax the arteries were applied locally to the carotid sinus and aortic pressoceptive areas

Experiments

Carotid Sinus Area In dogs anæsthetized with morphine chloralose the vagi aortic nerves were cut in order to limit the reflex regulation and homeostasis of arterial pressure to the receptors of the carotid sinuses The systemic

blood pressure was registered with a mercury manometer from a femoral artery The reflexes of carotid sinus origin were elicited by clamping and unclamping both common carotid arteries The drugs were applied to the walls of the arteries of the carotid sinuses by injection of 2 to 4 ml of their solutions into the space surrounding both carotid sinus areas

Experiments by Heymans and co workers (1950, 1951, 1952, 1953) showed that adrenaline, noradrenaline, diacetyl *adrenaline thiosulphonic acid, synephrine, ephedrine,* hydroxyphenylamino propanol (Aramine) hydroxytrypta mine (serotonin) and vasopressin applied locally to the carotid *sinus provoke a marked reflex fall of the systemic arterial pressure and a reduction or suppression of the hypertensive* reflexes normally provoked by decrease of intracarotid sinus *pressure*

The phenomena induced by local application of the drugs to the arterial walls of the carotid sinus are due to a stimula tion of the carotid sinus pressoreceptors Indeed section of the carotid sinus nerves when the arterial pressure has been lowered by local application of the drugs provokes an im mediate and very marked rise of the systemic arterial pressure

Fig 1 represents the curves of a typical experiment with noradrenaline

Local carotid sinus application of adrenolytic drugs such as *N* (2 bromo ethyl) *N* ethyl 1 naphthalene methylamine hydrobromide dihydroergotamine, ergotamine, dibenamine and *hydergine*, suppresses or reverses *the effects of locally* applied adrenaline and noradrenaline to the carotid sinus areas (Heymans *et al*, 1951 1952 1953 De Vleeschhouwer and Martini 1953)

It should be mentioned that Heymans and co workers (1933) and Moniz de Bettencourt (1935) had shown earlier that adrenaline lowers *the threshold of the carotid* sinus pressoreceptors and Palme (1936 1944) observed that local application of adrenaline to the carotid sinus areas of the rabbit hare and dog, provokes *a fall of systemic blood pressure*

Section of the carotid sinus nerve induced a rise of arterial pressure thus showing that the pressoceptors were stimulated Palme however attributed this action of adrenaline to a direct stimulation of the pressoceptors behaving as chemoceptors

Meurer (1949) also observed that local application of

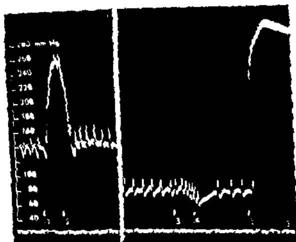


Fig 1 Dog—Blood pressure from femoral artery Vagi cardio-aortic nerves cut

(1–2) Clamping and unclamping of common carotid arteries Hypertensive reflex induced by lowering intracarotid sinus pressure (Between I and II) Local application of 25 μg noradrenaline to carotid sinus areas Fall of systemic arterial pressure from 140 to 80 mm Hg (II) About ten minutes after I (3–4) Clamping and unclamping of common carotid arteries No hypertensive reflex (5) Section of both carotid sinus nerves Marked hypertension from 80 to 300 mm Hg

adrenaline to the carotid sinus of man induces a fall of arterial pressure This author also attributes the action of adrenaline to a stimulation of carotid sinus chemoceptors

Our experiments further showed (Heymans *et al* 1950 1951 1952 1953) that drugs such as papaverine and benzyl imidazoline in doses which will relax smooth muscles when applied locally to the wall of the arteries of the carotid sinus

induce a reflex rise of systemic arterial pressure Fig 2 represents the curves of a typical experiment with benzylimidazoline

Landgren, Neil and Zotterman (1951) confirmed our observations on cats with adrenaline noradrenaline and vasopressin, and also showed that local carotid sinus application of these drugs elicits a very definite increase in the pressoceptors' impulse traffic Sodium nitrite administered locally to the carotid sinus areas causes, on the contrary, a marked reduction of the pressoceptor fibres' activity

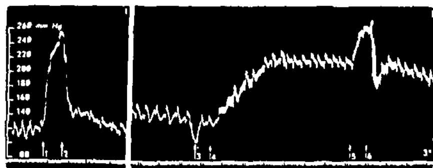


FIG 2 Dog —Blood pressure from femoral artery vagi aortic nerves cut

(1) Clamping of common carotid arteries (2) Unclamping of common carotid arteries (3–4) Local application of 0 25 ml 1 per cent benzylimidazoline to carotid sinus areas Rise of systemic arterial pressure from 140 to 2.0 mm Hg (5 6) Clamping and unclamping of common carotid arteries

Our experiments (Heymans and Delaunois 1951) demonstrated further that adrenaline and noradrenaline acting on the isolated carotid sinus preparation of dogs induce a contraction of the carotid sinus arterial walls, an increase of their pressure response and a decrease of their distensibility

From these and the previous experimental observations it was concluded that drugs contracting the arterial walls of the carotid sinus increasing their intrinsic tension and decreasing their distensibility cause a stimulation of the receptors of the carotid sinus nerves This stimulation

induces reflexly a fall in the systemic arterial pressure and decreases or suppresses the hypertensive reflexes normally provoked by decrease of blood pressure in the carotid sinus. Drugs relaxing the arterial walls of the carotid sinus and increasing their distensibility decrease the stimulation of the pressoceptors and thus induce a reflex rise of the systemic arterial pressure.

Landgren (1952) concluded from his experiments on the isolated carotid sinus preparation of cats that local application of adrenaline causes a contraction and a decrease of distensibility of the carotid sinus wall at low intrasinusal pressure ranges, but an increased distensibility in the region of the physiological intrasinusal pressure curve (80 to 180 mm Hg).

Our experiments on dogs (Heymans *et al.* 1953*b*) showed however that the same circulatory reactions induced by *local* carotid sinus application of noradrenaline occur *in vivo* at different *physiological* or non physiological intrasinusal pressure ranges. In these experiments a method (Fig 3) was *used* by means of which a constant pressure *may* be maintained *in both* carotid sinuses and intrasinusal pressure variations *may* also be elicited.

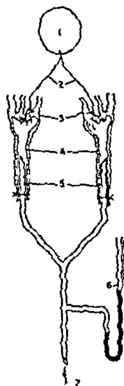


Fig 3. Diagram of method used for carotid sinus preparation (1) Vasomotor centres (2) Carotid sinus nerves (3) Carotid sinus (4) *Venous balloon* in carotid sinus (5) Cannula joining balloon and common carotid artery (6) Manometer for recording pressure in balloon and carotid sinus (7) Tube connected with pressure device and recorder

Fig 4 represents the results of a typical experiment *showing that local* application *of* noradrenaline to *the* carotid sinus areas when a constant intrasinusal pressure of 200 mm Hg is maintained induces a reflex fall in the systemic arterial pressure from 160 to

induce a reflex rise of systemic arterial pressure Fig 2 represents the curves of a typical experiment with benzylimidazoline

Landgren, Neil and Zotterman (1951) confirmed our observations on cats with adrenaline, noradrenaline and vasopressin, and also showed that local carotid sinus application of these drugs elicits a very definite increase in the pressoceptors' impulse traffic Sodium nitrite administered locally to the carotid sinus areas causes, on the contrary, a marked reduction of the pressoceptor fibres' activity

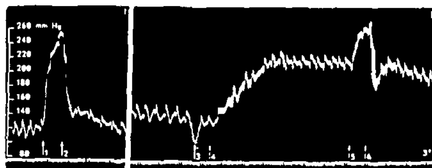


FIG 2 Dog —Blood pressure from femoral artery vagi aortic nerves cut

(1) Clamping of common carotid arteries (2) Unclamping of common carotid arteries (3–4) Local application of 0 2, ml 1 per cent benzylimidazoline to carotid sinus areas Rise of systemic arterial pressure from 140 to 220 mm Hg (5 6) Clamping and unclamping of common carotid arteries

Our experiments (Heymans and Delaunois 1951) demonstrated further that adrenaline and noradrenaline acting on the isolated carotid sinus preparation of dogs, induce a contraction of the carotid sinus arterial walls an increase of their pressure response and a decrease of their distensibility

From these and the previous experimental observations it was concluded that drugs contracting the arterial walls of the carotid sinus increasing their intrinsic tension and decreasing their distensibility cause a stimulation of the receptors of the carotid sinus nerves This stimulation

markedly increases the distensibility of the aorta over most of the pressure curve (0–200 mm Hg)

As our previous experiments (Heymans and Delaunois 1951) on the isolated carotid sinus preparation were performed at low intrasinusal pressure ranges a new series of experiments

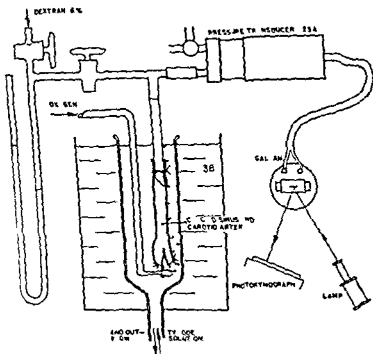


FIG 5 Apparatus used for measuring the pressure response of the isolated carotid sinus preparation

was performed (Heymans and Delaunois 1953) at different intrasinusal pressure ranges The following method (Fig 5) was used The efferent arteries of the carotid sinus are ligated and the cephalic end of the common carotid artery is connected with a Statham pressure transducer The ligated carotid sinus the segment of the common carotid artery and

100 mm Hg and a suppression of the hypertensive reflexes provoked normally by lowering intrasinusal pressure

Emmel and Smith (1952) have *demonstrated on isolated* perfused segments of arteries of dogs, that adrenaline induces an increased pressure response due to contraction of the

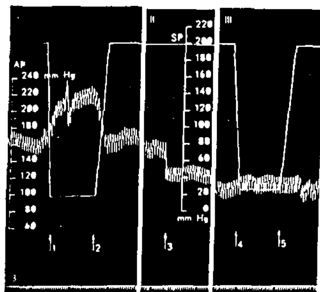


FIG 4 Dog —Carotid sinuses prepared according to method shown in Fig 3 Blood pressure from femoral artery (A I) Pressure in venous balloon and carotid sinuses (S I)

(1–2) Lowering and raising intrasinusal pressure Hypertensive and hypotensive blood pressure reflexes (3) Local application of 20 μg noradrenaline on each carotid sinus while maintaining constant intrasinusal pressure at 200 mm Hg Fall of systemic arterial pressure from 160 to 100 mm Hg (4 5) Lowering and raising intrasinusal pressure No response in systemic arterial pressure

arterial wall Alexander (1953) measured the pressure volume diagrams of isolated segments of the thoracic aorta in dogs and observed that local application of adrenaline produces a marked constriction of the aorta at low pressure ranges (0–100 mm Hg), but no significant change in volume at high pressure ranges He concluded however, that adrenaline

These experiments demonstrate that contraction increase of pressure response and decrease of distensibility of the arterial wall of the carotid sinus preparation are provoked by noradrenaline at different constant internal pressure ranges

The same observations were made with adrenaline

Benzylimidazoline induces on the contrary, a decrease in internal pressure and pressure response due to a relaxation and increase of distensibility of the carotid sinus arterial wall (Fig 7)

These experimental observations give further support to our conclusions that drugs inducing a contraction of the

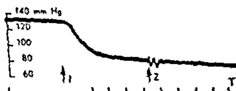


Fig Action of benzylimidazoline on pressure response of the isolated carotid sinus preparation
(At 1) Benzylimidazoline in concentration 2 × 10 followed by a decrease of internal pressure-response.

carotid sinus wall decrease their distensibility increase their resistance to stretch and thus increase the stimulation of the pressoceptors by the intrasinusal pressure provoking a reflex fall in the systemic arterial pressure Drugs relaxing the carotid sinus arterial wall induce the opposite reactions and thus cause a reflex rise in the systemic arterial pressure

Aortic Area Experiments by De Vleeschhouwer Martini and Calhauw (1953) in our laboratory showed that local application of adrenaline or noradrenaline to the aortic arch pressoceptive area also provokes a reflex fall in the systemic blood pressure (Fig 8)

Summary and Conclusions

The different series of experiments show that the state of contraction and tension and thus the resistance to stretch

the pressure transducer are filled with 6 per cent dextran solution at different constant internal pressures of 10 to 200 mm Hg The internal pressure response variations are registered by means of a mirror galvanometer connected to

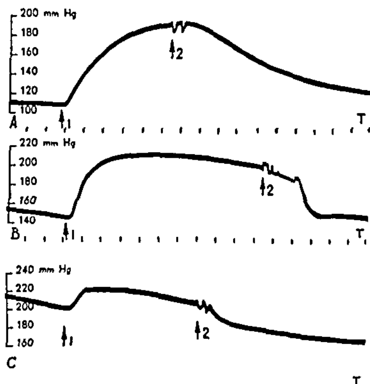


Fig 6 Action of noradrenaline on pressure responses of the isolated carotid sinus preparation at different intrasinusal pressure ranges

(At 1) Noradrenaline in concentration [illegible] × 10[illegible] followed by a rise of internal pressure responses (At 2) Washing out with Tyrode solution and return to previous levels of internal pressure responses

the pressure transducer Different drugs were added to the oxygenated Tyrode solution in which the isolated carotid sinus preparation was immersed

Fig 6 represents typical increases of pressure responses induced by noradrenaline at different constant levels of intrasinusal pressure

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DISCUSSION

PICKERING Supposing that we envisage some mechanism such as you suggest as the cause of essential hypertension how can we find out whether it is so or not? Some years ago Kissin Rothschild and I tried to find out to what extent the carotid sinus mechanism still was active in patients with essential hypertension We compressed the common carotid low in the neck below the sinus and recorded the changes in blood pressure and pulse rate We compared that with pressure on the femoral artery which we took to be an artery of roughly equal size and also with pressure on the neck Both those gave rises of blood pressure but the rise through compression of the carotid artery was greater The reflex did work in essential hypertension but the crux of the matter is what is the size of the response? Is there in man any better way than that of judging the way in which the sinus is responding to changes in intravascular pressure?

HEYMANS In patients there is no really objective method for the control of blood pressure regulating mechanisms Compression of the

of the arterial wall where the sino aortic pressoceptors are located, are the primary factors affecting these receptors which regulate and moderate reflexly the systemic arterial pressure These findings emphasize the fundamental role of the bio logical conditions of the sino aortic wall in the reflex regula tion and homeostasis of blood pressure, and suggest that decrease of tone and resistance to stretch of the sino aortic arterial wall could be the primary mechanism of hypertension

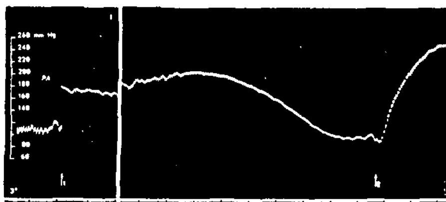


FIG 8 Dog anæsthetized with morphine-chloralose

(I A) Femoral arterial pressure (At 1) Section of both carotid sinus nerves (Between I and II) Local application to aortic arch area of 5 ml 1/5000 noradrenaline (II) Arterial pressure decreased from 205 to 100 mm Hg (At 2) Section of vagi aortic nerves induces rise of arterial pressure from 100 to 2_0 mm Hg

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dogs show that local application of noradrenaline to the arterial wall of the presso-receptor areas induces a decrease of arterial pressure

BEIN With regard to the hypertension produced by different pharmacological means a curious modification of sensitivity in the carotid sinus occlusion reflex has been found (Bein H J 1947 *Helv physiol Acta* 5 169 Miescher I 1951 *Arch int Pharmacodyn* 85 333) After injection of renin the occlusion reflex is greatly augmented In contrast to this the pressor reflex is practically abolished or is greatly diminished after an elevation of blood pressure produced by adrenaline The infusion of noradrenaline results in a slight augmentation of the pressor reflex to about the same extent as in the normal variation of blood pressure Renin thus seems to have some special action on the reflex mechanism of the carotid sinus this effect being the opposite of the action of injected adrenaline Whether the point of attack is central we do not know

NEIL Is it not rather dangerous to use the carotid occlusion test as a test of sinus nerve sensitivity?

BEIN One can for example also use Koch's set up i e perfusion of the isolated sinus with nerves intact and about the same relationship will be found

GADDUM Can Prof Heymans tell us what would happen if you put the carotid sinus in a little box and applied pressure *to it from outside* so that it is compressed although its volume is not raised Is it possible to do that?

HEYMANS We did not perform such experiments for technical reasons Compression of the carotid sinus from outside *may indeed* also stimulate nerve fibres But experiments by Koch and our observations show that lowering the pressure in the carotid sinus under 50 mm. Hg induces a deformation of the arterial wall towards the interior and provokes a stimulation of the presso-receptors and a reflex decrease of arterial pressure just as if the internal pressure in the carotid sinus were raised deforming the arterial wall towards the *outside* These observations show that the presso-receptors of the carotid sinus are responding to the deformation and tension of the arterial wall induced by pressure and not to the pressure itself

GADDUM They respond to stretch but if you make the muscle so that it cannot be stretched then they don't respond?

HEYMANS The tension and resistance to stretch of the arterial wall are indeed more important than the deformation of the arterial wall for the stimulation of the carotid sinus receptors

GADDUM So you cannot tell us what would happen if you apply the pressure from outside?

HEYMANS According to experimental observations by Hauss Asteroth and Kreuzinger increase of pressure from inside does not stimulate the carotid sinus receptors if deformation and resistance to this deformation of the carotid sinus arterial wall are counteracted from outside

MARTINI From the clinical standpoint your explanation is very good for older patients but it is difficult to conceive it operating in young men with hypertension

common carotid arteries induces a rise in systemic blood pressure but we cannot say if the reaction is more or less marked than normally

NEIL There is a paper by Kezdi in the *Archives of Internal Medicine* in January of this year (1953) he injected procaine into each carotid sinus in normal individuals and in hypertensive patients and measured the systolic and diastolic response and the heart rate In the essential hypertensive patients he found that there was a considerable response and he suggests (although it does not seem to follow from the records he gives) that the rise is rather less in essential hypertension This he ascribes to decreased stretchability ' as he calls it of the carotid sinus which is therefore doing rather less work at a higher mean pressure than would be the case in the normal individual

PICKERING Was he anæsthetizing the nerve?

NEIL Yes he was injecting quite large amounts of procaine into the vicinity of the carotid bifurcation

PAGE Isn t that the work that was done in Volhard s laboratory?

NEIL Yes

PAGE That is an incredible piece of work I don t understand it at all In the first place how did he get any normal human beings to allow both their carotid sinuses to be injected? In the second place how do you inject the carotid sinus in normal people? The third thing is the rises in blood pressure were phenomenal and with an injection *only on one side*

NEIL He did two sides in his last paper

PAGE Two sides would seem more likely to give a result than one but certainly these results in human beings are quite unlike any de buffering in dogs—fascinating if true!

NEIL And that despite the fact that the aortic nerves are still intact

PAGE There is another piece of work—I wonder what you think about it—in which the distensibility of the isolated carotid sinuses was measured Hypertensives were said to be less distensible I was un impressed with the type of controls used

HELLER I wonder whether an electrophysiological approach would be possible?

NEIL I should think perfectly possible If we took renal hypertensive dogs for instance it would be very simple to do action potential recordings from the carotid sinus nerve itself

HILLER It would be less hazardous than the experiments just described

HEYMANS Since in clinical essential or renal hypertension the blood pressure rises to a higher level and remains there it seems that the buffer mechanisms of arterial pressure are not working normally This disturbance of blood pressure homeostasis could of course occur at different points But as experimental observations show that such a disturbance shifting the blood pressure to a higher level occurs if the tension and resistance to stretch of the sino aortic arterial wall (where the receptors of the buffer nerves of blood pressure are located) are decreased we suggest that this could be the primary mechanism of hypertension Indeed preliminary experiments on renal hypertensive

PATON So that when a muscle contracts it pulls on the nerve ending and excites it

HEYMANS de Castro showed that the arterial wall of the carotid sinus has a peculiar anatomical structure It seems quite pos ible that the receptors the contractile elements and the connective tissue are in series

FLOYER To what extent do changes in the electrolyte composition of the fluid perfusing the carotid sinus have an effect on its response?

HEYMANS At the moment we have only applied drugs to the arterial wall of the carotid sinus

NEIL With regard to changes in electrolyte composition if you drop the calcium level to say 4–5 mg /100 ml. you get a noticeable increase in sensitivity and vice versa if you increase the calcium but I don t think that these effects are important physiologically

FLOYER Do changes in sodium and potassium concentration have any effect?

NEIL Potassium of course is a very powerful stimulant but I haven t tried the effect of small changes of potassium concentration such as may occur in the intact organism.

HEYMANS Would it not be possible that in young hypertensive patients the biological conditions of tension and resistance to stretch of the arterial wall are also decreased?

MARTINI With alteration in the vessels?

HEYMANS It is quite possible that before anatomical alterations occur in the vessels changes in the biological condition of the arterial vessels may occur Information concerning the mechanisms and factors involved in the maintenance and regulation of the intrinsic tone and resistance to stretch of the arterial wall is very scarce Observations by Cannon and Bacq and by Schmiterlow showed that the arterial wall contains adrenaline and noradrenaline One may suggest that they may play a rôle in the regulation of the tone and distensibility of the arterial wall where the receptors regulating and maintaining blood pressure are located Other factors may of course also be involved

PATON Your evidence seems to imply that the receptors in the carotid sinus are in series with contractile elements in the sinus It should be possible to see this histologically Has it been looked for?

HEYMANS I don t think histologists can give us information about changes in the biological conditions of the arterial wall if no anatomical changes are present

NEIL de Castro claims that the endings are solely in the adventitia and that there are no endings in the media Palme (1943) and Koster and Tschermak in about 1903 claimed to find endings in the media—but this latter work was done of course before we had any knowledge of the physiological function of the sinus region

If we examine the electroneurogram of the sinus nerve (cut centrally) before and after the local application of adrenaline to the corresponding sinus there is an obvious increase in the baroceptor discharge This occurs despite the fact that there is no alteration in the mean arterial pressure following such application because the sinus nerve is cut and reflex effects are thereby prevented from occurring The sinus wall contracts after application of adrenaline and the increase of baroceptor discharge which is particularly well shown by the small baroceptor fibres suggests that these nerve endings may be in series with the muscle fibres of the media The histologists must give us a bit of help there I should say however that if I perfuse the carotid sinus with solutions containing adrenaline up to concentrations of 10 ⁴ I cannot influence the action potential discharge in any way and if I stimulate the sympathetic supply to the sinus wall which undoubtedly causes a contraction of it that does not modify the reflexes which are produced So I should not have thought that actual changes of contraction of muscle in the wall of the sinus such as might occur in physiological circumstances would be important Of course degenerative changes that occur in the media might very well affect the changes of stretch

PAGE May I ask what you mean by in series ?

NEIL I take it to mean muscle fibre and then nerve ending embedded in connective tissue then another muscle fibre etc arranged circularly in the arterial wall

with a normal blood pressure The *first* of these consists of that period prior to the development of hypertension or any overt sign of sickness Even at this stage it becomes apparent that one is not dealing solely with an acquired condition the frequency of a family history and of family groups with an unusual tendency to arterial hypertension lends weight to the significance of hereditary patterns and the concept that some fundamental defect renders certain individuals more susceptible than others There is considerable evidence pointing toward variations in pressor response and disturbances in salt and water metabolism in patients with essential hypertension as compared to normal subjects These include differences in vascular reactivity (Hines 1951) in the effect of deoxycorticosterone on the blood pressure (Perera and Blood 1947) in the degree of weight loss and diuresis following salt withdrawal (Perera and Blood 1946) and in the excretion of sodium after thiocyanate administration (Pines and Perera 1952) Thomas and her associates utilizing the test of twenty four hours of salt restriction found that a certain percentage of normals behaved in similar fashion to uncomplicated hypertensives (Thomas Howard and Isaacs 1949) This group showed a higher incidence of familial hypertension overnutrition high normal blood pressure or vascular hyper reactivity than did the group which responded normally The story of one patient has been reported in whom an increase in arterial tension following deoxycorticosterone (as well as the failure to lose weight under the stimulus of rigid salt restriction) was evident prior to the appearance of the elevated blood pressure of hypertensive vascular disease (Perera 1951) Furthermore three of 25 young normals given deoxycorticosterone four of 25 placed on salt restriction and seven of 26 receiving thiocyanate reacted as did patients with essential hypertension Will these hyper reactors develop the disease? It has already been determined that they are more likely to have a positive family history These observations raise the possibility that an abnormal blood pressure may be but a manifestation of

HYPERTENSIVE DISEASE WITHOUT HYPERTENSION

GEORGE A PERERA

IN the state of Vermont they tell the tale of the lost traveller from the city He stopped for directions at a small farmhouse and received the usual instructions "the right turn after crossing the bridge bear left at the fork, then look for a white house with green shutters with a watering trough and a horse with a lame foreleg " The farmer paused suddenly and remarked To tell the truth if I were going to I I wouldn't start from here "

Whenever one deals with hypertensive vascular disease attention is focused immediately on the elevated arterial tension It is after all, the direct consequence of the increased peripheral resistance—the physiological explanation of the disorder The sphygmomanometer has become one of the most popular diagnostic instruments from its rather erratic *readings many physicians follow the progression of the disease* gauge its prognosis, and finally employ the blood pressure measurement as a definitive index of the efficacy of various procedures and drugs

I wonder sometimes whether this is the correct avenue of approach Should we heed the farmer s advice and make an entirely fresh start from another direction? If there are other roads they must not be ignored And so I propose that any consideration of humoral and neurogenic factors in hypertension should include all the evidence even the possibility that a disease can exist in the absence of its principal manifestation that manifestation from which it derives its name

During the lifetime of a patient with hypertensive vascular disease there may be as many as three periods associated

in heart size or alterations in renal function during such a period these may be due after all to the pre-existing organic disease or be the sequel to a drop in arterial tension or differences in cardiac function However in six instances to date (three after a myocardial infarction one after a cerebral thrombosis two after sympathectomy) we have seen papill œdema and retinopathy appear during this period of sustained normal blood pressure when it had been absent prior to the episode While hypertension was apparent all of these patients had documented essential hypertension with sub sequent proteinuria none of them had evident complicating or intracranial disease on clinical grounds they were all in the early malignant phase of their disease two have died with confirmation of the diagnosis of the accelerated phase with arteriolar necrosis Examination of the ocular fundi was made through dilated pupils by a single observer with at least three observations before the episode and one two weeks or more after during which interval no papillœdema hæmor rhages or exudates were seen It has been suggested that the mechanism of malignant hypertension is closely related to blood pressure levels particularly to increases above some critical level for each patient (Pickering 1952) There is no doubt that levels of arterial tension are generally—but by no means always—higher in this stage of the disease and that drugs sodium restricted regimens or surgical procedures which lower the blood pressure are associated usually with regression of retinopathy Nevertheless, the appearance of papillœdema and retinopathy in the absence of hypertension poses further questions regarding the mechanisms involved

These clinical observations relating to hypertensive vascular disease without hypertension require further docu mentation and provide few answers If some metabolic disturbance precedes the onset of an elevated blood pressure and if abnormal responses persist when a previously-existent hypertension has been removed temporarily, then attention must be given also to mechanisms other than those related primarily to arteriolar vasoconstriction If a complication

hypertensive vascular disease and that further search be made for some disturbance in advance of the first outward signs

The *second* period without hypertension is one which may occur at any time after the appearance of hypertensive disease At least 15 per cent of uncomplicated hypertensive patients, relaxed and rested in bed, will show temporary decreases in arterial tension to values less than 140/90 mm of mercury in from one to twenty one days This may take place even after twenty or more years of sustained disease Looked upon generally as a phenomenon of the early phases of the disorder, and commonplace at this stage, its frequency even after long established disease has been overlooked What of these subjects during their period of rest? Is their disorder—with its possible humoral and neurogenic components—still present? If vascular hyper reactivity existed beforehand, in the experience of our group it has been found that they retain their abnormal pressor patterns Unlike normals, the addition of large amounts of sodium chloride to the diet of individuals whose hypertension has disappeared after rest will produce an elevation of blood pressure Similarly, the daily administration of 5 10 mg of desoxycorticosterone acetate subcutaneously will be followed by a prompt rise in arterial tension We can only conclude that the *transitory release of generalized arteriolar constriction* does not obliterate all signs of an associated abnormality

There is yet a *third* period in which hypertension may vanish The majority of patients with hypertensive vascular disease will suffer eventually at varying rates and with variable areas of involvement, from organic complications referable to the heart or blood vessels Some will sustain a myocardial infarction a cerebral vascular accident, or as the result of the progression of their disorder be subjected to a bilateral thoracolumbar sympathectomy Any of these events may be followed in a small percentage of cases by weeks or months during which blood pressure values remain within the normal range One cannot interpret the development of new thrombotic or occlusive phenomena or changes

been elevated above normal At least the majority of the values recorded were within normal limits and it would be difficult to assume that the mean blood pressure could be anything but lower than the blood pressures obtained before the complicating factor

PICKERING What do you call normal?

PERERA For our purposes diastolic values less than 90 were regarded as normal

PAGE May I ask you Mr Chairman whether you think that the retinopathy is due to the preternaturally high blood pressure? In other words do you think that the vascular lesion in itself is due just to stretching of the vessels by increased diastolic pressure?

PICKERING I have always thought that the retinopathy is a consequence of the vascular disturbances associated with hypertension and that the focal lesions the hæmorrhages and exudates were probably due to focal disturbances in blood flow probably due to organic arterial lesions. And I have considered that the papillœdema and retinal œdema were a consequence of the level of intra-cranial pressure which was also probably in part determined by the level of arterial pressure But of course these cases are flat against it so far as they go

WILSON Dr Perera did you measure the CSF pressure in the patients who developed papillœdema after the blood pressure fell to normal?

PERERA No we did not

PAGE. Prof Pickering you measured the cerebrospinal fluid pressure didn t you in a series of cases? Taylor Corcoran and I found many cases with renal hypertension and many with malignant hypertension with normal cerebrospinal fluid pressures

PLATT We have found the same that although the cerebrospinal fluid pressure is very often elevated it is by no means always so at any rate at the time of our observations

I don t suppose Dr Perera had any opportunity of measuring cardiac output but presumably the actual height of the blood pressure may fall either because the peripheral resistance is lowered as we think happens perhaps with sympathectomy and with the use of hypotensive drugs or because cardiac output fall There may surely be a very big difference in the peripheral vascular resistance in those two situations It is a question which some day ought to be investigated

PERERA We did not measure the cardiac output

PAGE Malignant hypertension without retinopathy brings up the whole question of how you define malignant hypertension clinically I suppose it exists without retinopathy but we don t see it We don t see it because we feel that the diagnosis is not justified in the absence of retinopathy In other words we exclude it by definition Have you seen cases of hæmorrhagic necrotizing arteriolitis proven at autopsy without retinopathy or better who have never had grade 4 retinopathy We have all of course seen many patients in whom the eyegrounds have cleared up but autopsy evidence still showed the presence of necrotizing arteriolitis

PICKERING Not proven at autopsy but in some of the kidneys we

can develop after the disappearance of hypertension, at least in the accelerated phase, then we must re evaluate our concepts of pathogenesis We must ask ourselves whether the mere lowering of blood pressure by whatever means, is a sufficient therapeutic objective Note the disappearance of headache and the improvement in retinopathy which may follow sympathectomy even in those cases in which hypertension is not modified That the majority of methods of apparent therapeutic benefit also lower the blood pressure may be only a partial reason for their effectiveness In fact, the resultant decrease in blood pressure may be an associated phenomenon and one might raise the question that these therapies operate primarily on some neurogenic or other basis Perhaps the road travelled by those with hypertensive vascular disease does not always start, progress or even terminate with high blood pressure

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DISCUSSION

PICKERING I think you said that in the cases you described at the end of your talk although the blood pressure was normal papilledema and retinopathy appeared Did renal function decline and after that did some of them show arteriolar necroses?

PERERA Only two were followed until death and these two *did* have progressive renal damage Autopsy in one revealed extensive arteriolar necroses In the others the observations were made over a period of months and we do not have complete data on changes in renal function during that period

PICKERING The retinopathy was *bilateral?*

PERERA Yes One can raise the question that there may have been unrecorded periods during the day when the blood pressure might have

the level of blood pressure alone and call that hypertensive cardio-vascular disease that is a mistake almost childish in nature If we use the term essential hypertension unguardedly we may be including all manner of hypertensions with different mechanisms And I am sure you are right that there are vascular lesions without hypertension as proved experimentally But I couldn t accept for instance the fact that experimental neurogenic hypertension is entirely different from some types of essential hypertension I don t believe that the early concept that the hypertension was due purely to increased cardiac output is correct For instance if you use agents that slow the heart in the neurogenic hypertensive the blood pressure does not go down to normal which it ought to do if it were due only to an increased minute output The experimental neurogenic hypertensive does have an increase in peripheral resistance

PERERA Did you measure the cardiac output or did you assume the output change from the pulse rate change?

PAGE We measured the output Actually doing the measurements of the output again has not shown very much of an increase

FLOYER In what way was the blood pressure measured in these patients with papillœdema and retinopathy?

PERERA For the purpose of this study we have used both casual and what we choose to call resting values of blood pressure By the latter *I mean* multiple blood pressure readings taken in the morning with the patient lying relaxed in bed the lowest systolic and diastolic pressure recorded

FLOYER They *are all recorded* from the arm not a needle in the femoral artery

PERERA The usual sphygmomanometer cuff was employed only

FLOYER When a rat has what you *might call* malignant hypertension it becomes ill and may have fits if you kill it at that time and look at the tissues you find a lot *of acute* arterial lesions suggesting a sharp rise in blood pressure When you measure the blood pressure either on the tail or on *the foot* with or without anæsthesia you find frequently that it is subnormal Now *the animal* is in a shocked state and I have always imagined that these low blood pressure readings were due to the fact that *there is a* good deal of vasoconstriction in the vessels above the point of measurement Is it possible that in patients there is vasoconstriction of the arteries *even* with a big artery like the brachial artery which renders a *reading* by sphygmomanometer invalid?

PERERA I agree that such a possibility exists And yet many of us have the problem of trying to explain the few patients who after let us say a myocardial infarction without any apparent complications retain a normal blood pressure for long periods without any outward evidence of shock or failure

FLOYER I am thinking of the patients you described with papillœdema and normal blood pressure

PERERA The observations were made many weeks after the acute episode

FLOYER With the papillœdema still progressing?

have removed we have found necrotizing lesions in the absence of papilloedema

PERERA I have seen autopsied cases of malignant hypertension that had not previously been found to have retinal exudates or papilloedema

PAGE And the retinopathy never appears?

PERERA Not while under observation

PAGE We examine the eyegrounds routinely every week and it is amazing to me how few now we miss It can be as in toxemia of pregnancy that they come and go disappear and reappear I suspect that a good many of them are missed simply because they are not examined routinely I am still dubious about these rarities Not that I m convinced that it is the height of the blood pressure that causes necrotizing arteriolitis

PICKERING I wonder if anybody else has had any experience similar to Dr Perera in this matter?

GROLLMAN I am in complete accord with Dr Perera s viewpoint Although the cases that he reports are rare and exceptions to the general rule they cannot be ignored One must certainly take a broader view of hypertension than is usually done and not use this term as synonymous with an elevation in blood pressure as has unfortunately been done by clinicians as well as by experimentalists For example so called neurogenic hypertension induced experimentally by removing the buffer nerves in the dog resembles neither the disease as it is encountered clinically nor the disorder as it is induced by various manipulations of the kidney in the experimental animal It is true that in neurogenic hypertension the blood pressure may often be elevated but the hemodynamics of this condition are entirely different from what is seen in renal hypertension and the morphological alterations observed in renal hypertension are not encountered in the dog with neurogenic hypertension The use of the term hypertension or preferably hypertensive cardiovascular disease should be limited to a very specific and definable clinical and experimental syndrome and not used to designate every observed elevation in blood pressure There has been a tendency to consider anything that raises the blood pressure as a possible cause of hypertension and anything that lowers it as a potential therapeutic agent in the treatment of the disease It is to be emphasized that in addition to the rise in blood pressure observed in hypertensive cardiovascular disease there are definite pathological changes which are evident not only in the blood vessels but in the heart and smooth muscles of the body There are specific effects of the disease There is also a well defined hemodynamic pattern and other alterations in the organism all of which combined characterize hypertensive cardiovascular disease in addition to the rise in blood pressure The latter is only one manifestation of the disease it may be present under a variety of other conditions and as Dr Perera has indicated may under certain conditions not be present in the hypertensive patient nor in the hypertensive animal

PAGE I would agree with you that if we become preoccupied with

PERERA There is at least an association that cannot be denied from the incidence of myocardial infarction in hypertensives What provokes the augmented rate of arteriosclerosis in the average hypertensive is something I cannot answer

MARTINI But you see many cases of coronary thrombosis without any hypertension?

JIMÉNEZ DIAZ There are cases in which it is not possible to differentiate clinically between periarteritis nodosa subacute glomerulonephritis and malignant hypertension in the renal phase I think this is also the experience of Prof Platt The absence of hypertension inclines the diagnosis towards panarteritis Fahr reported some years ago cases of diffuse nephritis without hypertension and suggested the possibility of elimination in the urine of the hypertensive substance

PLATT Yes we have seen papillœdema in periarteritis nodosa a good many times but only with severe hypertension a diastolic pressure of 140 or something of that kind The exceptions to the general clinical rule that you do not get papillœdema unless the diastolic pressure is about 140 or over are seen in progressive cases of renal disease There you will see a retinopathy develop with considerably lower blood pressures diastolic pressures of 110 115 120

WILSON I feel that we tend to take one reading as evidence of the patient s blood pressure whereas of course it is only evidence of the blood pressure at that time But it is not uncommon to find what looks like papillœdema when the blood pressure is not excessively high I think the reason is that when papillœdema has been present for some time the appearances persist even when the malignant phase has temporarily subsided My view is that we should pay much more attention to the phasic character of malignant hypertension We are seeing it more and more in its early stages It is a phasic condition in which a period of malignant hypertension with obvious papillœdema may be followed by a quiescent phase in which there may be no albuminuria and the papillœdema to one who is accustomed to looking for it very regularly does in fact subside but leaves appearances that the non expert would still call papillœdema The physiological cup is obliterated the margins of the disc may be a little obscured but the vessels go straight across the disc and very often new vessels form the blood pressure may in this stage be stabilized at a lower level That of course would not explain the appearance of papillœdema for the first time under conditions of normal blood pressure Here perhaps the possibility of a transient phase of very high blood pressure is important. We know that in toxæmia of pregnancy a sudden detachment of the retina may occur and the patient become blind although when the blood pressure is taken it may not be excessively high But there must have been or at any rate I think it highly probable a phase of sudden vasoconstriction during that period Certainly in the experimental animal there are various observations which are extremely difficult to interpret if one assumes that the blood pressure has never risen above the recorded values In our original work we very occasionally observed severe necrotizing arteriolitis in animals in which the blood pressure

PERERA Papillœdema was not evident at least two weeks after the acute episode and only appeared at some time thereafter

MCMICHAEL So long as these patients were not in a dying state the sphygmomanometer readings from the brachial artery are valid I don t think you can explain that away I have also seen the appearance of papillœdema at quite moderately elevated levels of blood pressure We all know that without any treatment at all papillœdema will occasionally subside and disappear So it looks as though it is a super added episode on the course of hypertension and provided the patient survives long enough it can subside spontaneously It is certainly the easiest manifestation to ameliorate by any type of hypotensive treat ment Duke Elder (*Textbook of Ophthalmology* 1940 3 2718) states that once retinitis has subsided in hypertension it does not recur Can anybody tell me whether that is an acceptable generalization? We think there is some truth in it in that the retinitis does not return in the scarred areas

PICKERING I have met that statement but it is not right At any rate I have seen at least one patient who had a fullblown albuminuric retinitis during one of her pregnancies and that subsided after the pregnancy Of course that is the commonest example of the natural subsidence of one of these retinites She later went into the malignant phase and developed papillœdema she got no exudates but she did have quite definite papillœdema

MCMICHAEL But no exudate no true retinitis

PAGE I m afraid the retinitis has a very unhappy way of reappearing I think it should be added that toxæmia is the disease *par excellence* where retinopathy appears with a relatively low pressure We have all seen patients with blood pressures that were practically normal with marked retinopathy so there s no doubt that it appears in the absence of any marked hypertension But again that is a different disease

ROSENHEIM Papillœdema will occur in the course of cerebral œdema too If you have a high degree of hypertension and sodium retention then I think papillœdema will appear and may clear quite rapidly

JIMÉNEZ DIAZ We have seen some cases similar to those you have described but their interpretation cannot always be the same Some were cases of periarteritis nodosa in which it is possible to observe a malignant vascular and renal picture without hypertension although the blood pressure may be increased before or later In other cases there was a true essential hypertension with a malignant course in which the blood pressure fell as a consequence of cerebral thrombosis or myocardial infarction often a hypertensive patient would become practically nor motensive and the ECG would show the existence of myocardial infarct Sometimes we found the clinical picture of arteriolonecrosis without hypertension in patients with interstitial renal sclerosis (pyelonephritic)

MARTINI In periarteritis nodosa I have also seen retinopathy but never papillœdema Have you seen papillœdema in periarteritis nodosa?

JIMÉNEZ DIAZ No

MARTINI Do you believe Dr Perera that there is an obligatory correlation between coronary thrombosis and hypertension?

some initial retention of sodium and water but in the hypertensives the degree of sodium retention is roughly comparable to the normals Yet only the hypertensives show a rise in blood pressure which may persist after the salt and water retention has disappeared This observation does not exclude the possibility that there may be changes in salt and water metabolism that we cannot detect

records (usually at twice weekly intervals) showed no hypertensive reading As Dr Flover says the state of the animal in these circumstances is poor I do think we should pay more attention to the phasic character of malignant hypertension We had a rather static idea of it in the past and thought that once papilloedema had appeared the condition was permanent and steadily progressive

PERERA I quite agree with you and the reason for bringing these findings to your attention is to indicate that the relation of blood pressure to complications in hypertensive disease is an assumption not an established fact It may become a fact but there is no evidence as yet to explain every sign symptom manifestation or complication of hypertensive disease on the basis of blood pressure levels alone Therefore perhaps our minds should be open to the possibility that there may be neurogenic humoral or other mechanisms involved It is conceivable that they may operate through other means than on some mythical substance which is pressor in the circulation or in the body

WILSON Don t you think that once a certain blood pressure level is reached, the vessels may react abnormally that is to say a qualitative change in certain vascular fields may occur after the blood pressure reaches a certain point? Headache and papilloedema may disappear after sympathectomy and may not return even though the blood pressure rises again to the neighbourhood of the pre operative level as you have already said May not this be due to the elimination of blood pressure fluctuations which precipitate selective vascular spasm in such areas as the retina brain and even in the kidney?

HELLER Dr Perera on these pre hypertensive cases—your controls as it were—did you do any renal function tests sodium clearances or diodone clearances?

PERERA All the subjects the normals and the hypertensives were free of any demonstrable renal disease They had no proteinuria either concentrated urine successfully or excreted dye normally and had no nitrogen retention

LEDINGHAM Did you apply your tests with DCA and with the administration or restriction of salt in these particular cases who have returned to normotension after hypertension as the result of an infarct or other happening?

PERERA No but I must recall to you the patient I described in whom there was an immediate restoration of the hypertension after giving DCA when the pressure had fallen to normal

LEDINGHAM Have you also tested out the responses to salt in these particular cases?

PERERA We have The administration of large amounts of salt alone may restore the hypertension

ROSENHEIM That holds for patients whose blood pressure has become normal after a myocardial infarct does it?

PERERA Yes

CUNEST In the patients given DCA does the rise in blood pressure coincide with or follow the sodium retention?

PERERA In both normotensive and hypertensive patients there is

of de Molina and Wyss (1950) it is now known that in the vagus nerve there are A_β A_Δ, and B_1 fibres and of these the A_Δ fibres are of the greatest importance in vasopressor regulation The pressor effect of vagal stimulation is obtained with a pulse duration of 3 milliseconds though the frequency may vary between 10 100 cycles per second When the pulse duration is less than 0 5 milliseconds the effect is hypotensive Our technique for stimulation was as follows pulse duration

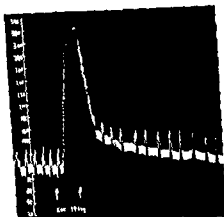


Fig 2 Effect of vagal stimulation in hypophysectomized dogs

of 5 milliseconds 50 cycles per second 3–6 volts stimulation for twenty seconds

One of the problems first attempted was the possible role of the endocrine glands in the increase of blood pressure Sattler (1940) held the view that stimulation of the vagus gave rise to the release of a hypertensive hormone from the pituitary body In the experiments carried out by the writers however it was proved that the hypertensive effect of stimulation persists in hypophysectomized dogs (Fig 2) The same effect can be induced after removing the adrenals kidneys and liver It is not therefore connected with the activity of any of these organs However the effect no longer appears consistently after cutting the cervical spinal cord and is greatly decreased by sectioning the splanchnic nerve (Fig 3)

INTERNAL SECRETION OF THE ARTERIAL WALL IN BLOOD PRESSURE REGULATION

C JIMENEZ DIAZ, P DE LA BARREDA, and A F DE MOLINA

ALTHOUGH many facts are known concerning the pathology of hypertension, present knowledge of the physiological

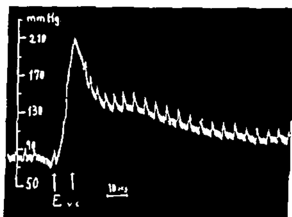


FIG 1 Effect on the blood pressure of stimulating the cut end of the vagus nerve at the neck level (dog)

mechanism of blood pressure regulation is not sufficient to explain the ætiopathogenesis of the disease in man

Some years ago we began to study possible factors in blood pressure regulation (Barreda and Jiménez Díaz, 1947, Jiménez Díaz *et al*, 1948, 1953) Our experiments were carried out on dogs and were based on the increase of pressure brought about by stimulating the central end of the vagus nerve sectioned at the level of the neck (Fig 1) In the dog the vagus and sympathetic nerves run side by side in the neck together with the carotid artery Thanks to the studies

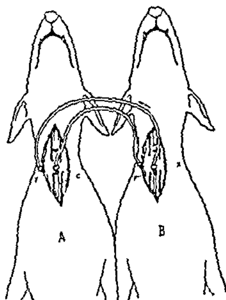


FIG. 4 Technique of crossed circulation in dogs

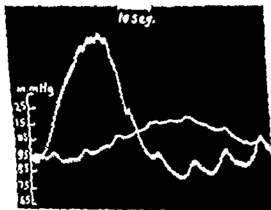


FIG. 5 Blood pressures of dogs with crossed circulation recorded simultaneously The rise of pressure occasioned by vagal stimulation in Dog A is seen almost at once in the record of Dog B

In the light of these facts we think that stimulation reaches the nerve centres and runs by sympathetic innervation towards the arteries

A further step was taken when we were able to prove for the *first time that this type of hypertension may be passed on to* another dog by means of a crossed circulation (Fig 4) As is seen in Fig 5 under such circumstances hypertension in the donor dog is seen to appear quickly in the recipient dog Such

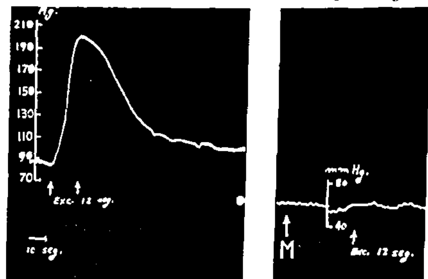


FIG 3 Effect of vagal stimulation before and after cutting the cervical sympathetic nerve (dog)

a transfer was confirmed in nearly 40 per cent of our experiments When hypertension was not transferred it could always be proved *that the recipient dog* was *only slightly* sensitive to noradrenaline and to direct vagal stimulation *This transfer* was subsequently confirmed by others (Binet and Burstein 1949, Taylor *et al* 1951) The fact that such a transfer may occur proves that on stimulation there is released into the blood stream a substance which does not originate from the organs excluded above When this substance circulates throughout the body hypertension occurs If this observation is not carried out with extreme caution

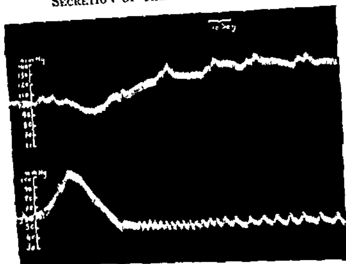


Fig 7

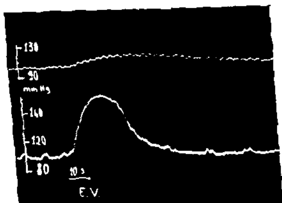


Fig 8

Fig 7 and 8 Stimulation of the vagus nerve in Dog A still produces a blood pressure rise in Dog B after elimination of *hæmodynamic factors*

some objections may be raised against it, the chief of which is the possibility of increased blood flow through anastomoses, because of the elevated pressure in the donor dog In order to avoid this possible source of error the following two additional procedures were used

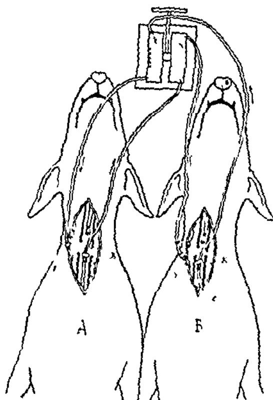


FIG 6 Crossed circulation in dogs using a mediating pump to regulate blood flow

(a) insertion of a pump between the two dogs (Fig 6) Under such conditions the transfer of the hypertension can still be shown (Fig 7)

(b) *Using flow regulating devices in both anastomoses* so that, the crossed circulation being open the pressure curves of both dogs may be parallel Under such conditions in which the hæmodynamic error is eliminated, the transfer is likewise seen to occur (Fig 8)

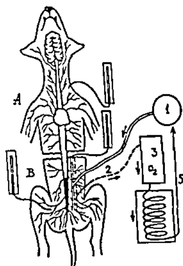


FIG 9a Diagram of "split dog" experiment The circulations in the two halves are independent

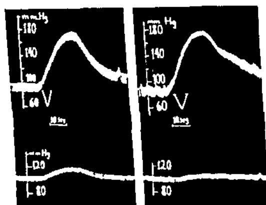


FIG 9b and c Blood pressure in two halves of the circulation in a "split dog" experiment Vagal stimulation (9b) causes a rise in both circulation but the injection of dibenamine (9c) into one circulation abolishes the rise in it alone

The fact that blood pressure changes are independent of volumetric factors may be confirmed in crossed circulation experiments if the recipient dog is "dibenaminized", stimulation of the vagus in the donor dog produces no effect in the recipient and the injection of adrenaline produces hypotension

In the light of these experiments the transfer of hypertension is a proved fact that points strongly to the release of a hypertensive substance into the circulation Early in our experiments we held the view that the place where such a substance is produced and from which it is released is the arterial wall itself In order to confirm this view we carried out what we called the "split dog" experiment A diagram of this is shown in Fig 9a The circulation in the anterior half of the dog is sustained by the heart and is completely independent of the posterior half, whose circulation is maintained by means of a pump at constant pressure This independence can be proved by injecting dye stuffs or adrenaline Under these conditions if the fluid circulating throughout the posterior part of the dog is oxygenated blood or a red blood cell suspension vagal stimulation is seen to induce hypertension not only in the anterior but also in the posterior part (Fig 9b) and the effect can be abolished by dibenamine (Fig 9c)

Taylor, Page and Corcoran (1951) confirmed most of our findings However they contend that the active substance is an internal secretion of the brain and not, as we believe, of the artery The results of our split dog experiments, however cannot be explained in this way since in these experiments the posterior part of the dog receives no blood that had previously gone through the brain Taylor and his colleagues also sometimes observed hypertensive effects of vagal stimulation after the spinal cord had been sectioned We sometimes obtained this result in our standard type of stimulation experiment but the rise was of a very different character, and we believe that it was due only to hæmodynamic factors We were unable to confirm their fundamental

(Fig 12) An inversion of the effect was never seen and therefore we concluded that the released substance was noradrenaline

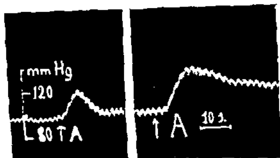


FIG 11 Hypertensive effect of arterial wall extract in the normal and cocainized dog

With extracts of arterial wall a noradrenaline effect is sometimes seen but much more frequently adrenaline is present whereas the main substance isolated from blood plasma during vagal stimulation is noradrenaline By means of paper chromatography we could isolate from the arterial wall adrenaline and noradrenaline defining its amount by von Euler s method which utilizes the effects on the fowl s rectal cœcum and the blood pressure of the cat Nevertheless the exact amount in the plasma has not as yet been estimated by us accurately perhaps because of the low content of our extracts

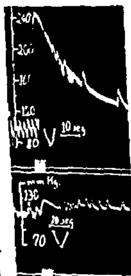


FIG 12 Effect of vagal stimulation in dibenamized dog

Raab (1943) proved that epinephrine and related substances were present in the arterial wall Schmiterlow (1948) verified that the effects were those of adrenaline and noradrenaline in such extracts Gaddum and Kwiatkowski (1938) observed that stimulation of

experiment using an isolated head perfused by the blood of another dog, to which we added the cutting of the cervical cord in order to avoid any action on the arteries In these conditions the results in nearly 40 experiments were negative (Fig 10)

In our opinion it is therefore, beyond reasonable doubt that the hypertensive substance is produced by the arterial

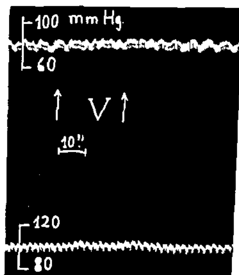


Fig 10 Isolated head of dog perfused with blood from another dog in which the cervical cord has been cut No rise of blood pressure occurs in the head on stimulation of the vagus nerve

wall We observed that some extracts of arterial wall were hypertensive the effect being increased by cocainizing the preparation (Fig 11) Our concept of the nature of this substance (which we initially called arterine) was influenced by the experiments with dibenamine which according to the work of Nickerson and Goodman (Nickerson and Goodman 1947, Nickerson 1949), abolishes adrenergic stimuli possibly by rendering the effector cells insensitive to adrenaline and sympathin We were able to verify that in dibenaminized dogs vagal stimulation no longer gives rise to hypertension

To sum up the stimulation of the arterial nerve supply results in the release from the arterial wall of hypertensive substances mainly noradrenaline This release is accompanied by a change in the enzymic activity of the arterial wall

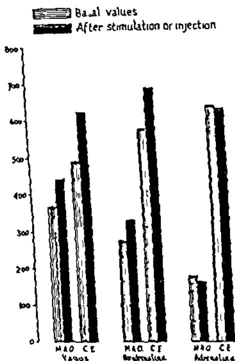


FIG 13 Comparison of the mono-amine-oxidase (MAO) and cholinesterase (CE) activity of arterial wall extracts (dog's abdominal aorta) before and after vagal stimulation and the injection of noradrenaline and adrenaline

We suggest that the artery is *not* a simple container but an active organ the mechanism of essential hypertension would then have to be considered from the different standpoint of a morbid condition resulting from arterial dysfunction It is likely that the constitutional basis of essential hypertension is a dys enzymic state The active role of the arterial wall

sympathetic innervation in the rabbit is followed by the production of a substance whose colour reactions in the ear vein correspond with those of adrenaline Cannon and Lissack (1939), working with arterial extracts, have observed hypertensive effects which are increased by cocaine and reversed by ergotoxine Peart (1949) has proved the presence of noradrenaline in the blood emerging from the spleen after stimulation of sympathetic innervation

It is difficult, as yet, to ascertain whether such substances are produced in the arterial wall itself or simply stored there and afterwards released Carr, Bell and Krantz (1952) have shown the presence of ATPase in the arterial wall of the dog This finding is extremely significant as it is now established (Bulbring, 1949) that adrenaline may be produced by methylation of noradrenaline by the suprarenal tissue in the presence of ATP In addition Thompson and Tickner (1951) have confirmed the presence of amine oxidase in the arterial wall Such enzymes cause adrenaline to break up and their presence in the iris and nictitating membrane has been proved by Burn (1952) We were encouraged by these findings to study with Villasante, the monoamine oxidase and cholinesterase activity of extracts of the arterial wall in rabbits and dogs both under basal conditions and after stimulation of the vagus and injection of adrenaline and noradrenaline The average values obtained are shown in Fig 18 The effect of adrenaline is not striking but those of noradrenaline and vagal stimulation are very similar

The reasons why we are inclined to suggest that noradrenaline is formed in the arterial wall itself are as follows

(1) The suppression of hypertensive effect in our "split dog" experiment, when the *posterior half is perfused* with saline
(2) The greater content of adrenaline than of noradrenaline *in the arterial* extracts
(3) The possibility of demethylation of adrenaline by tissues in the presence of choline, as was shown by Lockett (1952), this *process* is totally inhibited by dibenamine

then reversal to the previous normal level if the blocking with dibenamine is not complete you see a diphasic effect first hypo- and then hypertension This demonstrates that in the effect of vagus stimulation you have two factors first increase of adrenaline (induced by dibenamine) and then secretion of some substance similar to noradrenaline whose effect is suppressed but not inverted by dibenamine only if the dosage is sufficient. This substance is secreted even if adrenals pituitary, kidneys and liver are all extirpated

HEYMANS Did you remove the adrenal glands in the donor dog?

JIMÉNEZ DIAZ Yes and the transfer of the hypertensive substance persists It not only persists but if you dibenaminize the recipient dog and excite the donor at the time that the stimulation increases the blood pressure in the donor you obtain neither an increase nor a reversion effect in the donor Therefore this substance cannot be adrenaline but must be noradrenaline

HEYMANS A given amount of dibenamine may indeed invert the effect of adrenaline and block the hypertensive effect of noradrenaline

BARREDA No because we control the block in the donor dog before the connection is established between both dogs

JIMÉNEZ DIAZ We cannot make the mistake of influencing both dogs with dibenamine because the injection of one or the other is made with the crossed circulation closed The communication between the two dogs is only opened when we ascertain whether the effect in the injected dog is sufficient Then we leave the crossed circulation free for some minutes and do the experiments the other dog cannot be dibenaminized in under thirty minutes

HEYMANS Before concluding that a vasopressor substance is released by the arterial wall other possibilities have I think to be excluded—such as haemodynamic interferences and the presence of accessory adrenal tissue

Why should the vasopressor substance not be released at the sympathetic endings in the small blood vessels and other sympathetic innervated tissues? Are your experiments not related to the liberation of sympathin following the stimulation of adrenergic nerves? I don't see experimental data showing that such liberation really occurs in the arterial wall

BARREDA Perhaps you have not given attention to our split dog preparation in which the lower or posterior part of the dog is independent of the circulation in the other half in which are included the adrenals I remember you gave me the same suggestion some years ago There are other investigators for instance Dr Page who also find the same hypertension persisting after removal of the adrenals

HEYMANS But where are the experimental data showing that this pressor substance is really coming from the arterial wall?

When the isolated head of a dog is perfused from the circulation of another dog stimulation of the central end of the vagus sympathetic nerve of the isolated head may in some experiments induce a slight rise of blood pressure in the donor dog

JIMÉNEZ DIAZ Never!

in regulating blood pressure is a question which deserves continued attention

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DISCUSSION

HEYMANS Experiments in our laboratory have shown that stimulation of the central end of the vagus nerve in totally sympathectomized dogs no longer induces a rise of blood pressure The efferent pathways of the reflex rise of blood pressure provoked by central vagus stimulation are therefore located in the sympathetic No release of a vasopressor substance from the head could be observed after stimulation of the central end of the vagus sympathetic nerve in dogs

May I ask Prof Diaz if he has performed experiments stimulating the central end of the vagus in dogs after removal of the adrenal glands?

JIMÉNEZ DIAZ Yes we reported experiments in adrenalectomized dogs The hypertension after stimulation of the central end of the vagus persists of course with the same intensity We found also that total sympathectomy abolishes this hypertension Thus we think that the efferent pathway is in the sympathetic but the hypertension is mediated not only and not mainly by the adrenals If as we reported you stimulate the vagus in a dibenaminized dog you obtain first hypotension and

have been of the order of 0 1–0 $\mu\mu$g/g while in the spleen there would be something like 2–4μg/g showing that in such vascular regions the amount of such substance is very large compared with the big arteries

There is also one other question whether these arteries contain the sympathomimetic amines in the artery wall itself or whether they are bound to the nerves I think in Schmiterlow s experiments it was quite nicely shown that there is a good correlation between the number of nerves demonstrated histologically and the amount of pressor substance I think that fits in very well with the other experience we have had that these catechol amines mostly noradrenaline are due to the presence of postganglionic sympathetic nerves which we know contain large amounts of noradrenaline

With regard to the adrenal glands it is quite obvious from experiments in man that after complete adrenalectomy the excretion of noradrenaline in the urine is unaltered while the adrenaline practically disappears That would seem to show that the noradrenaline comes from another source presumably the adrenergic nerves I think that would account for the action which is the same kind as in Cannon s experiments where he stimulated the sympathetic nerves and there was a certain overflow of sympathin acting on the blood pressure or on the denervated heart

HELLER Have you tried umbilical artery extracts?

VON EULER We have tried placenta but did not find *anything* at all

BEIN We have compared in the cat the influence of various drugs with a different point of attack (namely a sympathicolytic Regitine a ganglionic blocking substance pendiomide a phthalazine derivative Apresoline and a recently isolated alkaloid from *Rauwolfia serpentina* Reserpine or Serpasil) on the blood pressure rise produced either by stimulation of the central end of the vagus *or* for comparison by adrenaline or by noradrenaline All *three blood pressure* increases were *of about the same* magnitude After sympathicolytic drugs there is an inhibition of this blood pressure rise which is similar to an inhibition of the noradrenaline blood pressure effect whereas the effect of adrenaline is *reversed* During the ganglionic block there is also an inhibition *of the effect* of the stimulus but in contrast to the sympathicolytic an *augmentation of the effect of* adrenaline and noradrenaline Thus we can confirm that the efferent pathway runs in the sympathetic system as it can be blocked by both sympathicolytics and ganglionic blocking drugs Now Apresoline acts in a somewhat different manner as the *effect of* the stimulus is inhibited to a *much* greater extent than the *effect of* adrenaline or noradrenaline (*Schweiz med Wschr* 19 3 83 336) This we think rather confirms Dr Page s findings that Apresoline inhibits somewhat specifically the effect of stimulation of the central end of the vagus although some peripheral sympathicolytic effect is *present* too

In contrast to the effect of these three *drugs* the *Rauwolfia* alkaloid Serpasil has no sympathicolytic and no ganglionic blocking activity In spite of this Serpasil inhibits the blood pressure increases due to

Heymans If the blood pressure of the donor dog is low stimulation of the central end of the vagus sympathetic nerve of the perfused head may induce some rise of pressure in the donor dog But this is a purely hæmodynamic phenomenon provoked by the vasoconstriction induced in the perfused head by stimulation of the sympathetic The same rise of pressure occurs in the donor dog if the arterial connections between donor dog and perfused head are clamped I agree with your conclusions that stimulation of the central end of the vagus sympathetic nerve does not release a vasopressor substance in the cephalic circulation

Barbeiro I agree with you about your result—but that is no objection to our research

Heymans I still think that these observations are an objection to your conclusions Why do the arterial walls of the cephalic circulation not release a vasopressor substance after stimulation of the sympathetic although according to your conclusions other arterial areas do it?

Jiménez Díaz We welcome your criticism But we have made an addition to the experiment devised by Taylor and his colleagues and we cut the cervical spinal cord under these conditions we never obtain an increase of blood pressure in the donor dog By cutting the spinal cord we abolish the secretion of the substance in the sympathetic innervated arteries of the head We thought indeed that the positive results of the other authors could be explained by the secretion in these artery walls as you suggest But under our conditions with a sectioned spinal cord that is no longer possible and indeed it was not possible to obtain any hypertensive effect

von Euler I think some of our experiments might throw a little light on some of the controversial points here To begin with the last one that not all arterial regions seem to be able to release the same amount of pressor substance I think that is true Because apparently the arteries of the head and especially the brain do contain much less noradrenaline than the rest of the body If for instance one makes extracts of the brain for noradrenaline one finds quite small amounts There is one exception which has been shown by Marthe Vogt that there is a lot of it in the hypothalamic region but that is a small region and the overall action is quite small Also I think it is established that extracts of arteries do contain sympathomimetic amines If I understood Prof Díaz correctly the extracts contained quite considerable amounts of adrenaline and yet the effect on the recipient dog was one of noradrenaline I could not quite understand the significance of that But the fact is that if one makes extracts of arteries say large arteries like the mesenteric artery or femoral artery of the dog they do contain a certain amount of adrenaline but on the other hand that amount seems to decrease the more carefully one prepares the arteries the more care in making these extracts the less pressor substance is found in them and the less adrenaline So it seems to have something to do either with nerves running on the outside of the artery or with chromaffin cell groups in the artery wall or in the vicinity of the artery wall Usually extracts of the large arteries contain relatively small amounts We have recently made extracts of various dog arteries and the amounts

DRUGS ANTAGONISTIC TO 5 HYDROXYTRYPTAMINE

J H GADDUM

A DRUG with a specific antagonistic action to 5 hydroxy tryptamine (5 HT) might be valuable in various ways It might throw light on the physiological function of 5 HT it might aid the identification of 5 HT in tissues and facilitate investigations devoted to other pharmacologically active substances in tissue extracts it might even be used in thera peutics I propose to give an account of a search for such an antagonist carried out by E Fingl H Hameed and myself during the last year or two (Fingl and Gaddum 1953)

When a comparatively large dose of 5 HT is applied to isolated ileum from a guinea pig the resulting large con traction does not last long but the muscle relaxes while the 5 HT is still in the bath Such muscles still respond normally to histamine or substance P but not to tryptamine An excess of tryptamine in the bath causes a similar specific desensitization of the muscle (Gaddum 1953a) These facts have already proved useful in the pharmacological analysis of tissue extracts (Feldberg and Toh 1953) but antagonists which themselves have large effects are clearly not perfect antagonists and a number of other substances have been studied

Specific antagonisms between drugs are commonly due to competition between them for the same receptor in the tissues This fact became generally known among enzyme chemists and pharmacologists about 1930 (Clark 1937) It became known to bacteriologists ten years later and has often led to the finding of competitive antagonists among the chemical relatives of active drugs We have therefore studied a number of synthetic substances which like 5 HT contain an indole nucleus Most of these were made specially for this

stimulation and augments the pressor response to adrenaline and noradrenaline (*Experientia* 1953, 9 107) As there is no sympathicolytic and no ganglionic blocking activity but a block of the stimulus which comes from the afferent side an interruption must have taken place between the afferent and efferent sides of the reflex arc It thus seems unnecessary to assume that a special humoral transmittor substance is released when the central end of the vagus is stimulated I don t know whether Prof von Euler will agree

VON EULER Ergotoxin does the same doesn t it? It blocks centrally

BEIN But it blocks at the periphery just as much

JIMÉNEZ DIAZ Our experience of the content of adrenaline and noradrenaline in the arteries measured by the same method as Prof von Euler s was that there is a very variable amount of adrenaline and noradrenaline depending first on the region the artery was taken from and secondly on other conditions that we cannot as yet ascertain The relation between adrenaline and noradrenaline is also variable but in general in the artery walls the proportion of adrenaline is much greater than noradrenaline about five times more adrenaline than noradrenaline On the other hand in the circulating blood there is about five times more noradrenaline than adrenaline This makes us think that perhaps the artery can synthesize noradrenaline—that is only a suggestion but it is possible In adrenal tissue in the presence of choline and without ATPase it is possible to demethylate adrenaline and form noradrenaline We speak only of the arterial wall but we are not in a position to say where in the arterial wall is the site of production

adrenaline Ergometrine has little or no antagonistic action either to 5 HT or to adrenaline

These results may be summarized by saying that several alkaloids derived from ergot are even more active as antagonists of 5 HT than as antagonists of adrenaline On the other hand some other drugs which are known to be powerful antagonists of adrenaline appear to have little or no action against 5 HT For example piperoxane (F 933 piperidyl methyl benzodioxane) in a concentration of 10^{-6} (1 mg /l) abolished the effect of adrenaline on the rabbit s ear without causing any change in the response to 5 HT

These experiments are still incomplete They have already proved helpful in the pharmacological analysis of tissue extracts They have told us nothing definite so far about the physiological functions of 5 HT Their results obviously cannot be applied yet to therapeutics Our most active antagonist produces temporary madness in very small doses It may be possible to find an antagonist to 5 HT without this effect on the brain On the other hand it is possible that the *5 HT in our brains* plays an essential part in keeping us sane and that this effect *of* L S D *is due* to its inhibitory action *on the 5 HT* in the brain If this is so it may perhaps be difficult *to* inhibit the action of 5 HT on arteries without causing insanity

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[Discussion of this paper was postponed until after the paper by Dr Erspamer —ED]

purpose by Messrs Glaxo Several of these substances are active and specific antagonists, but the most active substance we have found so far is lysergic acid diethylamide (L S D) which we owe to Messrs Sandoz This alkaloid which is made from ergot, has powerful effects on the human brain in a dose of 1 μg per kg (Stoll, 1947) Lysergic acid contains tryptamine as part of its molecule L S D in a concentration of less than 1 in 100 millions (10 μg /l) inhibits the action of 5 HT on rat's uterus while leaving the action of carbachol unchanged (Gaddum, 1953b) This tissue was found by Erspamer to be particularly sensitive to 5 HT We find that it is also particularly suitable for the detection of specific antagonism

However, L S D also specifically inhibits the vasoconstrictor action of 5 HT on the perfused ear of a rabbit In one experiment, for example, after a few preliminary trials, the ear was perfused with a solution containing L S D in a concentration of 10 μg /l This inhibited the response to 5 HT even when the dose of the latter was increased over 300 times At the same time the response to adrenaline appeared to be somewhat increased by the L S D The antagonistic effect to 5 HT developed slowly during an hour or more, and disappeared in an hour when the ear was once more perfused with a solution containing no L S D

Several other alkaloids from ergot have been tested Dihydroergotamine is known to be an active antagonist of adrenaline We have found it very active as an antagonist of 5 HT, though not so active or specific as L S D When a direct comparison was made in the same rabbit's ear with control doses of both adrenaline and 5 HT it was found that dihydroergotamine was more active against 5 HT than against adrenaline Under suitable conditions it was possible to diminish the response to 5 HT whilst leaving that to adrenaline unaffected but slightly higher concentrations abolished both responses

Ergotamine itself has similar properties to dihydroergotamine, but is rather less active against both 5 HT and

Normally the titration data may be considered sufficiently accurate when the 5 hydroxytryptamine content exceeds 0 1 µg per ml in serum and 0 2-0 3 µg per g in tissues For smaller amounts of 5 hydroxytryptamine other substances known as well as unknown, can cause disturbing interferences We can reveal the presence of these substances by blocking the 5 hydroxytryptamine action with dibenamine or gramine

Our main results are summarized in Tables I II and III The first two tables illustrate the 5 hydroxytryptamine (5 HT) content per ml of serum or per g of fresh tissue the last the 5 HT content per kg body weight

For comparative purposes some data are given on the 5 HT content in the intestinal tube of Ascidians in the skin of Amphibians in the hæmolymph the gut and the posterior salivary glands of Octopods as well as in the hypobranchial body of *Murex trunculus*

Table I shows that 5 HT is present in gastro-intestinal extracts of all the Vertebrates studied with the exception only of some groups of Fishes (*Teleostei* and *Cyclostomata*) In these groups the gastro intestinal mucosa is notoriously *lacking* in typical enterochromaffin cells which we consider to be the site of production and/or deposit of 5 hydroxytryptamine

This substance is also present in extracts of Ascidian intestine (presence of enterochromaffin cells) *lacking* in those of Octopod intestine (absence of enterochromaffin cells)

The 5 HT content varies conspicuously from one animal species to another and in the same species from one section to another of the intestinal tube

Still *more* conspicuous species differences are to be seen as Table II clearly *shows* in the 5 HT content of serum Also the individual variations are here as a rule very important and it *seems quite probable* that the serum 5 HT level is by *no means* constant in a single individual

From preliminary researches it appears that the serum of older persons contains a little less 5 HT than that of younger persons

QUANTITATIVE ESTIMATION OF 5-HYDROXYTRYPTAMINE IN GASTRO-INTESTINAL MUCOSA, SPLEEN AND BLOOD OF VERTEBRATES

V ERSPAMER

THE biological titration of 5 hydroxytryptamine (enteramine, serotonin) has been carried out on the isolated, atropinized œstrous uterus of the rat This test object offers the following advantages

(a) Conspicuous sensitivity to 5 hydroxytryptamine The uterine horn usually gives good responses down to dilutions of 1 in 100–200 million

(b) Negligible sensitivity towards many other active tissue or hormonal products Interference by acetylcholine, histamine adenosine compounds, callicrein, vasopressin, angiotonin, tyramine, octopamine, adrenaline noradrenaline bradykinin and pepsitensin may be practically ruled out under our experimental conditions and for the materials we examined Moreover, substance P of Euler and Gaddum, towards which the rat uterus shows a moderate sensitivity, does not seem to represent a source of error of any importance

(c) Prompt regression of the spasmogenic effect after washing out with fresh nutrient liquid, and rapid recovery of the original reactivity that is to say absence of tachyphylaxis

(d) Satisfactory agreement within certain limits of course, of the reaction to the dose of 5 hydroxytryptamine The error does not exceed 10 to 15 per cent

(e) Excellent resistance of the uterine smooth muscle

The results obtained by us agree very well with those obtained by other research workers (Reid 1952 Feldberg and Toh, 1953) with other test objects

Table II

Animal species	5-Hydroxytryptamine content as base			
	Serum (µg/ml.)		Spleen tissue (µg/g)	
	Mean value	Range	Mean value	Range
Humans				
newborn	0 07 (3)	0 06-0 09	—	—
20-23 years old	0 1 (14)	0 07-0 20	—	—
60-90 years old	0 09 (16)	0 03-0 15	—	—
Beef	1 48 (9)	0 61-2 60	7 80 (3)	6 1[illegible]- 8 91
Goat	2 18 (6)	0 7[illegible]-3 28	4 60 (2)	4 [illegible]0-4 00
Sheep	0 8[illegible] (4)	0 36-1 4[illegible]	3 80 (4)	1 48-0 80
Horse	0 41 (8)	0 2[illegible]-0 8	1 76 (3)	1 23 2 60
Ass	0 41 (3)	0 28-0 4[illegible]	3 10 (1)	—
Ho	0 [illegible]6 (5)	0 14-0 37	1 [illegible]3 (4)	0 82 2 [illegible]1
Dog	0 21 (10)	0 08-0 57	1 40 (7)	0 61 2 0[illegible]
Cat	3 80 ( )	1 24-7 40	8 40 (4)	5 74 10 49
Rabbit	3 53 (1[illegible])	1 80-5 [illegible]7	1[illegible] 60 ([illegible])	16 40-2[illegible] 50
Guinea pig	0 [illegible]1 (10)	0 07-0 27	1 06 (7)	0 [illegible]-1 6[illegible]
Rat	0 97 (3)	0 57-1 7[illegible]	[illegible] 80 (30)	—
Mouse	1 48 (10)	—	1 80 (10)	—
Hamster	0 37 (3)	0 3[illegible]-0 41	—	—
Urchin	1 84 (3)	0 76-2 78	1 80 (3)	0 8[illegible] 2 [illegible]8
Bat (*Rhinol phus ferrum equinum*)	3 56 (16)	—	18 90 (16)	—
Hen	[illegible] 7[illegible] (15)	2 [illegible]6-3 6[illegible]	1[illegible] 54 (7)	8 60-18 4[illegible]
Guinea hen	[illegible] 66 (3)	2 00-3 3[illegible]	—	—
Turkey	0 11 (3)	0 09-0 13	—	—
Duck	1 16 (1)	—	4 10 (1)	—
Pigeon	0 33 (7)	0 18-0 49	—	—
Sea mew	0 69 (1)	—	—	—
Stork	0 03 (1)	—	—	—
Tropid motus natrix	0 41 (37)	—	0 16 (37)	—
Testud graeca	0 01 (1)	—	0 01 (1)	—
Rana esculenta	0 18 (40)	—	0 08 (40)	—
Bufo bufo bufo	0 0[illegible] (16)	—	—	—
Scylliorhinus canicula	<0 02 (5)	—	<0 08 (5)	—
Scylliorh stellaris	<0 04 ([illegible])	—	<0 06 (2)	—
Torpedo marmorata	<0 0[illegible] (1)	—	<0 08 (1)	—
Ang lla vulgaris	<0 0[illegible] (5)	—	<0 0[illegible] (5)	—
Tinca vul aris	<0 04 (8)	—	<0 0[illegible] (8)	—
Imiurus calus	<0 0[illegible] (17)	—	—	—
Petromyzon planeri	<0 0[illegible] (4[illegible])	—	—	—
Petrom zon marinus	<0 03 (1)	—	—	—
Octopus vulg ris	<0 04 (3)	Haemolymph	—	—
Oct pus macropus	<0 04 (3)		—	—
Eledone moschata	<0 04 (3)		—	—

I parentheses is th nur ber of specimens from whi h the mean value w re btained.

In spite of the fact that we have sound reasons for believing that serum 5 HT originates from the enterochromaffin cells the 5 HT content of serum shows no relation whatever to that of the digestive tube

Table I

Species and number of specimens	5 Hydroxytryptamine content (μg/g)			
	Stomach	Small intestine ‡	Small intestine §	Large intestine
Dog (3)	5 20	3 70	4 30	2 80
Cat (3)	0 45	0 88	0 5[illegible]	1 10
Rabbit (3)	4 00*–0 85†	3 30	3 70	[illegible] 70
Guinea pig ([illegible])	1 40	5 00	3 40	0 70
Rat (10)	1 40	1 20		3 00
Mouse (10)	[illegible]	1 60		3 10
Bat (11)		1 60		
Urchin (3)	6 30	← 3 40 →		
Hen ([illegible])		4 90	4 [illegible]0	4 10
Pigeon (3)		← 1 10 →		
Duck (1)		3 10	4 10	3 60
Tortoise (1)		3 [illegible]0		
Toad (15)	[illegible] 20	← 1 60 →		
Bombinator pachypus (65)	1 00	← 0 75 →		
Scylliorhinus canicula (5)	0 60	← [illegible] 60 →		
Scylliorh stellaris (2)	0 30	← 2 30 →		
Torpedo marmorata (1)	1 3[illegible]	← 2 [illegible]0 →		
Amiurus catus (17)	0 30(?)	← < 0 40(?) →		
Anguilla vulgaris (5)	← 0 30(?) →			
Tinca vulgaris (11)	← < 0 20(?) →			
Petromyzon planeri (4[illegible])	← < 0 20(?) →			
Tethium plicatum (114)	← 0 5[illegible] →			
Ciona intestinalis (14[illegible])	0 3	0 20		
Octopus vulgaris (12)	← < 0 20(?) →			
Eledone moschata (1[illegible])	← < 0 20(?) →			
Discoglossus pictus	Skin			410–[illegible]40
Bombinator pachypus				700 1000
Hyla arborea				80–110
Bufo mauretanicus				110
Octopus vulgaris	Post salivary glands			420–512
Eledone moschata				720
Murex trunculus	Hypobranchial body			80 290

* = Stomach body
† = Stomach pylorus
‡ = Proximal half of the small intestine
§ = Distal half of the small intestine

Table III

	5 Hydroxytryptamine [illegible] fresh weight			
	Serum	Spleen	Gastro-intestinal tract	Total
Man	4 5	—	—	—
Beef	51 8	—	—	—
Goat	77 [illegible]	—	—	—
Sheep	20 7	—	—	—
Horse	14 3	—	—	—
Ass	14 3	—	—	—
Hog	9 1	—	—	—
Dog	7 5 (4 7)	8 6 (5 4)	111 0 (80 0)	157 5 (100)
Cat	133 0 (70 [illegible])	10 6 (5 8)	38 4 (0 5)	189 0 (100)
Rabbit	1[illegible]3 5 (83)	5 8 (1 6)	24[illegible] 0 (65 [illegible])	371 0 (100)
Guinea pig	7 3 (4 6)	1 6 (1 3)	14 [illegible] (91 1)	151 1 (100)
Rat	34 0 (7 1)	11 0 (8 9)	80 1 (84 0)	1[illegible]5 1 (100)
Mouse	51 8 (14 7)	15 6 (4 4)	[illegible]83 6 (80 0)	333 0 (100)
Hamster	13 0	—	—	—
Bat	[illegible]4 0 (53 3)	5 0 (13 8)	77 1 (32 0)	33 7 (100)
Urchin	84 4 (0 3)	8 5 (2 6)	[illegible]44 4 (77 1)	317 1 (100)
Hen	—	18 8	10 0	—
Duck	—	1 6	104 4	—
Discoglossus p[illegible] / B[illegible] [illegible]unctor	Skin			41 000 54 000 / 175 000 250 000
Octopus vulgaris / Eledone moschata	Post salivary glands			1 90 1570 / 2[illegible]80

The contrary may be said for spleen 5 HT, whose close relationship to serum 5 HT is beyond question. It is probable that the substance found in spleen has to be considered as of thrombocytic origin exclusively, being presumably enclosed in phagocytized thrombocytes or thrombocyte fragments.

Amphibian skin may contain enormous quantities of 5 HT or related hydroxyindolalkylamines, with quite normal levels of serum and gastro intestinal 5 HT. It is probable that skin 5 HT has nothing to do, in regard either to origin or to physiological significance, with the other localizations of this substance in the Amphibians.

The data collected in Table III offer for the first time a picture of the total 5 HT content in the body of several animal species, including the common laboratory species and illustrate the percentage distribution of the substance in gastro intestinal mucosa, blood and spleen.

We consider these data, like those on the distribution of 5 HT in the animal kingdom, of considerable importance in the interpretation of the biological significance of the entero chromaffin cells and of their secretory product. When trying to explain the function of 5 HT, the wide distribution of this product amongst the Vertebrates and the Ascidians should always be kept in mind.

Moreover, it is quite obvious that amongst the many biological actions possessed by 5 HT, the only ones which are to be taken as possibly "physiological" are those provoked by doses smaller than the total 5 HT content in the organism of the experimental animal.

These conditions seem to be fulfilled by our hypothesis which considers 5 HT as a hormone designed to control the function of the kidneys and, more particularly, intrarenal hæmodynamics (Erspamer and Ottolenghi, 1953).

Our point of view is strongly supported by the following observations.

(1) The antidiuretic action of 5 hydroxytryptamine, as a consequence of an afferent vasospasm, appears in the rat

ERSPAMER V and SALA G (1954) Brit J Pharmacol in press
FELDBERG W and TOH C C (1953) J Physiol 119 352
HELLER H (195) Ciba Foundation Colloquia on Endocrin 4 463
REID G (195) J Physiol 118 435

DISCUSSION

PAGE Dr Erspamer is to be congratulated on his very objective presentation of a very complicated subject I should add that Dr Twarog in our laboratory has used the *Venus* heart in measuring the 5 hydroxytryptamine content of various organs and generally seems to confirm what Dr Erspamer has said The *sensitivity of this preparation is* 10⁻⁹ molecular of 5 hydroxytryptamine I *believe you told* me that the rat uterus was 1 200 million how that compares I don t know but probably the rat uterus is a little more *sensitive* I *might* add that our measurements in the brain showed 0 1–0 30µg *so* there is a fairish amount in brain of dog and rabbit as compared with serum our figures for serum were 0 1–0 4 µg *so the* brain contains as much as serum does Another point is that the urine contains a fair amount of it as well If you infuse 5-hydroxytryptamine about 5 per cent of it appears in the urine the rest seems to be *non recoverable* I should add that there is at least in the *Venus* heart a certain amount of blocking some of the organs for instance the extracts of *heart* show a small blockade from substances associated with the *serotonin* in the extract so we are not too sure that the figures are always correct We were unable to detect any in plasma which may *mean* that there is only a very *small amount* present or that the method is not sensitive enough to pick it up

The only thing I would *disagree* on is the question of the effect of *this* substance on the kidney Dr Corcoran s experiments were almost uniformly negative in the sense that he found nothing to suggest *any* specific effect of serotonin I have not the slightest idea why there *should* be this disagreement and I think it will probably come out when all the figures are known on the clearances He has infused it injected it subcutaneously and except for the *painful* effect of the serotonin he did not get any striking *effect either* on the diuretic response or in changing clearance Patients don t like to have serotonin administered to *them* We use it in small amounts as a measure of neurogenic tone in man

ERSPAMER *I have* been experimenting with the mollusc heart since 1950 I agree with Dr Page that this preparation is often e g in the case of *Octopus* and *Helix* heart fairly sensitive *to* 5-hydroxytryptamine but unfortunately I do not find it *specific* enough for this substance I think it may be used only as a subsidiary test object in the quantitative estimation of 5 hydroxytryptamine in crude tissue extracts

Our diuresis experiments on hydrated rats were carried out over a six year *period* on about four thousand groups of four animals each 5-Hydroxytryptamine has *been regularly* antidiuretic starting from *subcutaneous* doses as low as 4 µg per kg of body weight Thus dose is one fiftieth of the dose which acts on the systemic blood *pressure*

after the subcutaneous injection of as little as 4 μg/kg of substance This quantity corresponds to one eighth of that present in serum, and to one thirtieth of that present in the entire organism

In the dog a drastic renal vasoconstriction may be provoked by the quantity of 5 hydroxytryptamine normally present in blood (see renal vasoconstrictor effect of the fresh defibrinated blood) But this quantity makes up only one twentieth of the total 5 hydroxytryptamine in the body

(2) The subcutaneous injection of the acetone extract obtained from 1 ml of rat serum per 100 g of body weight, causes in the hydrated rat a significant decrease in urine flow, quite similar to that obtained with 1 ml of genuine serum We believe, mainly on the basis of research carried out on rabbit serum that this effect may be, at least to a great extent, ascribed to 5 hydroxytryptamine (Erspamer and Sala 1954)

The unstable antidiuretic substance described by Dicker and Ginsburg (1950) and by Heller and Ginsburg (Heller, 1952) in rat serum is therefore, in our opinion, identifiable with 5 hydroxytryptamine

(3) Only a few groups of Fishes, the *Teleostei* and the *Cyclostomata* lack enterochromaffin cells and 5 hydroxytryptamine But, just in these groups, owing to the particular environmental osmotic gradient and the consequent necessity for reducing glomerular activity the glomeruli have undergone marked reduction in size and capillarity, and in many forms have disappeared entirely leading to the formation of an aglomerular kidney

In sharp contrast with that of *Teleostei* the kidney of the *Elasmobranchii* shows large and well developed glomeruli and, at the same time, the gastro intestinal mucosa contains beautiful enterochromaffin cells and abundant 5 hydroxytryptamine

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antimetabolite potency of this product on the rat uterus preparation but failed to obtain the same result on the rat diuresis test

Heller: I wonder if I might say something about the antidiuretic effect of hydroxytryptamine which Dr Erspamer mentioned. I think there are two pieces of work that have been done on the antidiuretic effect of this substance in dogs, both of these as yet unpublished. Sala and Abrahams and Pickford have done some experiments and both these groups of workers have found an antidiuretic effect in very small doses. However they do not quite agree in their conclusions in so far as Dr Sala thinks that with very small doses he gets a tubular effect, whereas Dr Pickford told me that even with the smallest doses she used there was only a vascular effect. So considering that Dr Erspamer has also found an antidiuretic effect in rats, there can be little doubt about the action of 5-hydroxytryptamine though its mechanism is perhaps not quite clear. Also you said, Dr Erspamer, that the doses which produced the antidiuretic effect are about one fiftieth of the amount present in the body. Judging by other antidiuretic substances that is a very large amount considering that the parallel amount of antidiuretic hormone is in the region of 10, so perhaps one must be a little careful in deducing anything as to the physiological effects of 5-HT.

Erspamer: Certainly. But the chemical constitution of 5-hydroxytryptamine is very simple when compared to that of the posterior pituitary antidiuretic factor. It is to be expected that the biosynthesis of 5-hydroxytryptamine acts very rapidly starting from the widely distributed L-tryptophan. Udenfriend and his associates succeeded recently in demonstrating the existence in the organism of two enzyme systems which assure on the one hand the *oxidation* of L-tryptophan to L-5-hydroxytryptophan (tryptophan oxidase) and on the other hand the decarboxylation of L-hydroxytryptophan to 5-hydroxytryptamine (5-hydroxytryptophan decarboxylase).

It seems therefore that the storage of the latter substance in the enterochromaffin cells could be easily and promptly renewed when exhausted. This point of view is corroborated by Udenfriend's experiments with radioactive trytophan.

Gaddum: Is the antidiuretic effect of 5-hydroxytryptamine antagonized by dibenamine?

Erspamer: Yes, when dibenamine is given two to three hours previously. When it is given simultaneously there is no effect.

Heymans: We could see an antidiuretic effect of serotonin in anaesthetized dogs.

Page: With no effect on blood pressure?

Heymans: The dose injected induced a rise of blood pressure. Barac also observed an antidiuretic effect with serotonin in dogs. This effect could be antagonized with ascorbic acid.

Pickering: Were the experiments you referred to, Dr Erspamer, in conscious or anaesthetized animals?

Erspamer: In conscious rats.

Pickering: And yours, Prof. Heller?

Heller: Conscious dogs in both cases, I think.

and 10 000–20 000 times smaller than the median lethal dose by the subcutaneous route

The opinion that 5 hydroxytryptamine antidiuresis may be ascribed to the pain producing effect of the substance seems to be untenable since 1 ml of a 1/100 000–1/1 000 000 solution of enteramine picrate does not produce more pain than 1 ml of distilled water and since dibenamine and the sympatholytic ergot alkaloids to which no analgesic action is attributed are capable of blocking the 5 hydroxytryptamine antidiuresis

The substance reduces the urine flow not only in the rat but according to Sala and Castegnaro also in the dog starting from subcutaneous doses of 40 μg /kg body weight To these two experimental animals we have to add human beings both normal and suffering from diabetes insipidus Two cases of this disease responded consistently to the subcutaneous injection of a crude enteramine extract of *Octopus* We are now going to repeat the experiments with pure 5 hydroxytryptamine

Finally I should like to emphasize again that the antidiuretic effect is much more conspicuous after subcutaneous injection than after intraperitoneal or intravenous administration Why this occurs I don t know but it is possible that it has something to do with the uptake and release of 5 hydroxytryptamine by the platelets We have to keep in mind that in the platelets the substance is protected against any enzyme attack and is thus allowed to reach the site of its physiological action unchanged

PAGE You might be interested in some results we have had with 5 aminoindole the one that Woolley and Shaw were proposing as an antimetabolite for 5 HT We found it blocked pressor action in quite large doses but strangely enough did not block the depressor actions of 5 hydroxytryptamine As regards hypertensives we gave 10 g /day for twenty days to a hypertensive patient and it did nothing to the blood pressure I may say that our results did not agree with Woolley and Shaw for we did not get an effect by mouth in dogs This was with a sample of nitroindole which Woolley sent me I talked with him about it and then he also found that he did not get a reversal with this sample of material He prepared another sample and both of us have now found the same reversal which confirms his original observation but leaves entirely unexplained why one sample which was analytically pure failed to act I wonder if Prof Heymans remembers that he did the original work in 1932 in demonstrating that ergotamine blocked the action of serum vasoconstrictors long before serotonin had been discovered?

CADDUM I certainly remember that and I m sorry I didn t mention it

ERSPAMER I agree with Dr Page in considering the antimetabolite potency of 2 methyl 3 ethyl 5 aminoindole and of 2 3 dimethyl 5 aminoindole very small Quite recently however Woolley and Shaw have synthesized another 5 hydroxytryptamine analogue which seems to be considerably more active I refer to 2 methyl 3 ethyl 5 dimethyl aminoindole (Medmain) We have been able to confirm the greater

5-hydroxytryptamine into the rat we have detected on urine chromatograms at least two indole spots, one very faint, probably consisting of a hydroxytryptamine itself, and the other much clearer consisting of some breakdown product of 5-hydroxytryptamine, presumably 5-hydroxyindoleacetic acid.

VON EULER. Is there any difference in the output of 5-HT in the urine of hypertensives and of normals?

PAGE. We cannot answer that at the moment. It seems that everybody agrees that it is antidiuretic except us. We simply have found nothing remarkable about serotonin's actions on the kidneys.

PICKERING. Does it have an effect on the chlorides?

ERSPAMER. I have not seen any effect.

PAGE. Curiously enough, when we infused the material into anæsthetized dogs, in most dogs we had a fall in pressure, and the only effect on glomerular filtration rate Corcoran got during that time was probably attributable to the hypotension. This is quite different from the single injection, when you get the amphibaric effect on the arterial pressure.

GADDUM. What do you think is the cause of the hypotension? I know that in cats one important cause is constriction of the pulmonary artery.

PAGE. It has that immediate effect, yes. But Dr. Marrazzi measured the action potentials in the ciliary nerve produced by shocks delivered to the preganglionic oculomotor nerve and found during the initial fall in dogs a transient blockade lasting possibly twenty seconds only, and then reappearing. We thought the initial fall in blood pressure was therefore due to two things: the depressor cardio-pulmonary reflex plus this transient blockade. There is a later fall in pressure due to inhibition of vasoconstrictor tone.

BEIN. According to the work of Schneider in the United States there is after injection of *serotonin* an activation of certain peripheral afferent receptors, for example the lung stretch receptors, the effect found being similar to that produced by *injection* of veratrine. This could be taken as an indication that serotonin has some reflex action.

GADDUM. That effect has also been recorded in this country, but the receptors are probably not identical with stretch receptors.* 5-HT also causes vasoconstriction in perfused lungs.†

BEIN. But do you also have a vasoconstriction *in situ* in the rat? Because with Rein's Thermostromuhr we did not find a marked reduction of flow in the pulmonary arteries after serotonin.

GADDUM. I found a large rise of pressure in the pulmonary artery *in situ* coming on in about three seconds and disappearing very rapidly.

PAGE. Did you find the same thing in dogs?

GADDUM. I haven't tried.

HEYMANS. It is different in dogs.

PAGE. Yes, and the neurogenic hypertensive dog behaves like the cat.

ERSPAMER. In favour of the renal point of attack of 5-hydroxytrypta-

* Mott, J. C. and Paintal, A. S. (1953). *Brit. J. Pharmacol.* 8, [illegible]

† McCallum, J. H., Hebb, C. O., Silver, A. and Swan, A. A. B. (1953). *Quart. J. exp. Physiol.* 38, [illegible]

ERSPAMER 5 Hydroxytryptamine is certainly present in dog s blood in concentrations active on the kidney vessels When we perfuse the kidney vessels with defibrinated blood they constrict powerfully It has been seen by Starling and Verney

HEYMANS Defibrinated blood is not normal blood however Is hydroxytryptamine present in normal blood?

ERSPAMER I think so

HEYMANS Is hydroxytryptamine present in the tissues or released on extraction?

GADDUM I can tell you something about that In cat s blood if you collect it very carefully so as not to break up the platelets and then centrifuge it the plasma contains very little But if you collect it in an ordinary glass vessel and wait two to three minutes the plasma contains a lot I think it is contained in the platelets

PAGE That is our experience too Platelets certainly liberate serotonin and reasonably quickly too

ERSPAMER In two cases of Werlhof s disease with a very low platelet count we have found in serum a hardly detectable amount of 5 hydroxytryptamine

HEYMANS Is hydroxytryptamine released in some conditions from the tissues into the blood?

ERSPAMER I think it must be released from the enterochromaffin cells into the plasma and thence taken up by the thrombocytes Also *in centrifuged heparinized hen s blood 5 hydroxytryptamine is contained* only in the white cells layer and I expect it must be the same in all vertebrates The circumstance that 5 hydroxytryptamine is not detectable in the serum of some fishes possessing enteramine may depend upon the absence or rarity in these species of blood cells analogous to thrombocytes

PRATT Dr Page in your experiments with the infusion of 5 hydroxytryptamine you said that about 5 per cent came out in the urine Is there any evidence for the presence of 5-HT in normal urine? That would perhaps answer some of these questions?

PAGE Yes there is

PRATT Obviously the objections raised about collection of blood are very valid There is some evidence I think that using calcium citrate as an anticoagulant has a different effect from the use of heparin I certainly found that one had to use enormous amounts of heparin to prevent the disappearance of serum vasoconstrictor I got the impression that heparin was far more important—if you used much more than you needed to *maintain an anticoagulant action—than any other factor*—than collection into siliconed tubes or anything else I think that the urinary excretion would provide much more information about its presence in the circulation in man than collection at the present time of blood

PAGE We found it in the normal urine of both man and dogs

GADDUM There is evidence that tryptamine appears in the urine partly as indoleacetic acid the plant hormone

ERSPAMER Following the subcutaneous injection of 5 mg/kg of

CHEMICAL SCREENING METHODS FOR THE DIAGNOSIS OF PHÆOCHROMOCYTOMA

M GOLDENBERG

THE varying clinical syndromes observed in phæochromocytoma can be grouped as follows

(1) Paroxysmal hypertension (adrenal sympathetic syndrome)
(2) Persistent hypertension mimicking essential or malignant hypertension
(3) A combination of hypertension hypermetabolism and glycosuria and
(4) Persistent hypermetabolism or hyperglycæmia coexistent with intermittent hypertension

The understanding of these widely varying syndromes has been helped by the demonstration that these tumours harbour two agents adrenaline and noradrenaline in varying proportions (Holton 1949 Goldenberg Faber Alston and Chargaff 1949) Other variables are the rate of secretion of the tumours and secondary endocrine and vascular (smooth muscle) changes

A fraction of the cases studied one third according to Green (1946) and one fourth in our own series (Goldenberg Aranow Smith and Faber 1950) exhibits this classical type of phæochromocytoma which is characterized by paroxysms (sometimes termed adrenal sympathetic syndrome) They are comparable to the effects of a rapid intravenous injection of a pharmacological dose of adrenaline and (or) noradrenaline Observations on cases which combine paroxysmal hypertension with persistent hypermetabolism or persistent hyperglycæmia suggest that the resting secretion of these tumours may be not negligible but rather insufficient to

mine I should like to emphasize again that the substance is absent in some groups of fishes the *Teleostei* and that just in these groups the glomeruli have undergone involution In the *Teleostei* the intestinal mucosa is very rich in cells which are indistinguishable from the typical enteramine containing enterochromaffin cells both in their morphology and the behaviour of their granules using the common histological staining methods These cells however which we call pre enterochromaffin argentophil cells are completely lacking in 5 hydroxytryptamine They may be tentatively interpreted as enterochromaffin cells which have lost enteramine during the evolution of the bony fishes

Why do only the *Teleostei* (and not the *Elasmobranchii* in which the glomeruli are well developed) lack typical enterochromaffin cells and 5 hydroxytryptamine? It seems to me important

GADDUM Yes, I agree But I feel that 5-HT may have more than one function

ERSPAMER It is possible But the distribution of the substance in the animal kingdom must be considered when explaining the function

GADDUM Yes it is important that it occurs in such animals as the octopus

PICKERING The question has not yet been fully answered whether 5-HT is present in tissues as such or whether it appears in the process of extraction or incubation

PAGE I think the answer is nobody knows

ERSPAMER It seems probable however that the substance pre exists in the cells in a form similar to that present in the extracts The specific granules of the enterochromaffin cells after formalin fixation, give exactly the same chemical colour reactions as do *in vitro* 5 hydroxytryptamine solutions treated with formalin Moreover it is possible by colour reactions to detect the substance also in the fresh unfixed enterochromaffin cells These observations of course do not exclude the possibility that 5 hydroxytryptamine may be loosely bound in the living cell to some carrier substance

GADDUM It must be bound somehow but it is very easily extracted with acetone The position is the same as that of histamine

ERSPAMER Still more closely I believe the same as that of adrenaline and noradrenaline in the chromaffin tissue

PAGE It must be filtrable also otherwise it probably wouldn t be in the urine

ERSPAMER We have also tested in preliminary experiments the enteramine content in the blood of 25 hypertensive human beings There is no augmentation of hydroxytryptamine the level is normal or even subnormal

PICKERING Then at present we can probably write it off as having nothing to do with the cause of hypertension

PLATT It may be irrevelant to this meeting but what is the effect of giving hydroxytryptamine to schizophrenics? No doubt that s been tried

GADDUM I don t think it has I shouldn t like to give it Presumably to get an effect you would have to give it intravenously and I haven t had it intravenously

adrenergic blocking agents in the diagnosis of this disease (Goldenberg and Aranow 1950) Although the incidence of false negative tests does not exceed 10 per cent (*cf* below) a direct method which excludes the variability of the pharmacological response is preferable The increased urinary output of noradrenaline and adrenaline in phæochromocytoma seemed to constitute an ideal basis for such a method Urinary excretion of adrenaline and other sympathomimetic amines after ingestion was first demonstrated by Richter (1940) The occurrence of catechol amines in normal human urines was first suggested by Holtz Credner and Kroneberg (1937) and confirmed by von Euler and Hellner (1951) The diagnostic value of the increased urinary output of adrenaline and noradrenaline was first described by Engel and von Euler (1950) and confirmed by Goldenberg and Rapport (1951) At this stage the method necessitated after suitable urinary adsorption procedures the use of a bioassay It was our endeavour to replace the bioassay procedure by chemical methods suitable for the diagnosis of phæochromocytoma and for the quantitation of adrenaline and noradrenaline The chemical methods studied all depend on the photofluorescence of reaction products of adrenaline and noradrenaline These substances adrenolutine and the corresponding noradrenolutine (Lund 1949*a*) are formed from adrenaline and noradrenaline respectively with adrenochrome and noradrenochrome as intermediates

The methods studied in the course of our work have included the following

(1) Preparation of urinary extracts by adsorption on precipitated aluminium hydroxide
 (1*a*) Bioassay
 (1*b*) Paper chromatography
 (1*c*) Photofluorometric evaluation
 (1*d*) Quantitation of adrenaline and noradrenaline
(2) Rapid screening procedure using column adsorption
(3) Pharmacological tests

produce pressure changes This may well be due to the fact that metabolic effects are caused by much smaller doses of adrenaline than those required to produce hypertension

More often phæochromocytoma is associated with persistent hypertension The easily obtained adrenergic blocking effect in these cases suggests that this hypertension is due to continuous secretion of adrenaline and (or) noradrenaline by the tumour

Correlation of clinical and chemical data in this group (Goldenberg *et al*, 1950) indicated that small tumours which contained noradrenaline predominantly (90 to 97 per cent) and not more than a total of 80 mg of this catechol amine gave a syndrome mimicking essential hypertension with unimpressive metabolic features As the total amount of noradrenaline in the tumours increased additional evidence of hypermetabolism and hyperglycæmia was found although noradrenaline caused these to a much lesser degree than an equal amount of adrenaline When the predominant catechol amine in the neoplasm was adrenaline hypertension hypermetabolism, hyperglycæmia and tachycardia occurred

A surprising observation was that patients with tumours containing large quantities of adrenaline may, at times present a clinical picture indistinguishable from that of essential hypertensive vascular disease, with normal heart rate, absence of hyperglycæmia absence of metabolic disturbance as indicated by normal basal oxygen consumption, and a negative or equivocal response to Benzodioxane This suggested that persistent hypertension in patients with phæochromocytoma is not due at all times to the presence in the circulation of sufficient quantities of noradrenaline or adrenaline to cause hypertension by direct cardiovascular action, i e of the sort seen in the acute infusion experiment This concept is well supported by the finding that hypertension may outlast the removal of the tumour for a varying length of time (Goldenberg *et al* 1950)

It is this last mentioned phase of "non humoral" hypertension in phæochromocytoma which limits the value of

line in twenty four hours The other cases in this group reproduced the pattern of hypertension with hypermetabolism and hyperglycæmia

The excretion calculated per single ml of extract (=100 ml urine) did not exceed 6 μg in the essential hyper tensive group and ranged from 42–250 μg noradrenaline equivalent in the first phæochromocytoma group These observations facilitate the use of chemical screening methods on a constant urine volume without introducing a volume factor

In the group of paroxysmal hypertension due to phæo chromocytoma in seven cases the urinary excretion of catechol amines ranged from 190–1530 μg noradrenaline equivalent One case excreted 110 μg adrenaline which is far in excess of the adrenaline excretion found in normoten sives and in cases of essential hypertension In none of these cases did severe or long lasting paroxysms occur at the time of urine collection It is noteworthy that in one case one mild and short paroxysm only was observed at the time of urine collection with a twenty four hour excretion of 1530 μg noradrenaline equivalent There was no objective or sub jective evidence of a paroxysm during the urine collection in three cases

If the urinary catechol amine excretion in this second group of phæochromocytoma normotensive between paroxysms is compared with the normotensive group the excretion appears four to 30 times higher if compared with the essential hyper tensive group two to 15 times higher

The urinary excretion calculated per ml extract (=100 ml urine) ranged in the paroxysmal phæochromocytoma group from 20 μg noradrenaline and 20 μg adrenaline to 100 μg noradrenaline and 100 μg adrenaline

Of the 16 cases of phæochromocytoma studied 15 proved positive when studied by bioassay on the cat's blood pressure alone

(1b) The catechol amine content of the urine extracts has been studied with the aid of ascending one dimensional paper

(1a) The urinary extracts were obtained by adsorption on precipitated aluminium hydroxide, desalting and concentration *in vacuo* (usually 100 1) These extracts were taken up in 0 01 N HCl and quantitated by bioassay (For details of the method Goldenberg, Serlin, Edwards and Rapport 1954) This method is a modification of the adsorption procedure of von Euler and Hellner (1951) The urinary extracts were assayed on the cat's blood pressure and the values expressed as noradrenaline equivalents

The urinary excretion in twenty four hours ranged from 14–41 *μg noradrenaline equivalent in the normotensive* group (13 subjects) and 7–100 μg noradrenaline equivalent in the group of essential hypertensive vascular disease (35 subjects) In more than two thirds of these patients the urinary excretion was within the normotensive range

The 16 cases of phæochromocytoma studied showed a varying clinical picture They can be subdivided into two main groups nine cases of persistent and seven cases of paroxysmal hypertension (with normal blood pressure between attacks) with and without paroxysms during the period of urine collection

In the first group the urinary catechol amine excretion is far above that of the normotensive and essential hypertensive group It ranges from 600–2700 μg noradrenaline equivalent in twenty four hours Since the excretion of catechol amines in the essential hypertensive group ranged from 7–100 μg, with an average of 27 μg in 35 cases the differentiation appears clear cut If one uses 100 μg as the upper limit (only once encountered in our group of 35 cases of essential hypertension) the catechol amine excretion in our first phæochromocytoma group (persistent hypertension) is 6 to 27 times higher

In three of these cases, the clinical picture was that of essential hypertensive vascular disease A differentiation was impossible in one of these who showed false negative responses to Benzodioxane, Regitine and histamine The urinary catechol amine excretion of this case was 675 μg noradrena-

of mixtures of noradrenaline and adrenaline by differential oxidation at pH 3 and pH 6 5

In our experience the differentiation of phæochromocytoma from essential hypertensive vascular disease can best be achieved by this type of photofluorometric evaluation of the urine extracts obtained by adsorption on precipitated aluminium hydroxide This method does not permit a separate quantitation of adrenaline and noradrenaline in the urine extracts but is limited to obtaining a total fluorescence value at pH 6 0 using a Coleman 12 B photofluorometer and PC 2 secondary and B1 B2 and B3 primary filters The highest specific readings for adrenaline and noradrenaline are obtained with the B 3 filter which passes part of the 436 mμ and all of the 405 mμ line B 1 readings (this filter passes the 365 mμ line) and B 2 readings (this filter passes the 436 mμ line) are necessary to screen out non specific fluorescence in the urine extracts In phæochromocytoma B 3 readings are always higher than those of B 1 or B 2 and the ratio B 1 B 2 lies between 0 7 and 2 0 which represents the ratios for adrenaline and noradrenaline respectively

The B 3 readings for 0 02 ml urine extract (100 1) ranged from 138 to 1694 in our 16 cases of phæochromocytoma These readings do not parallel the noradrenaline equivalent per ml values obtained by bioassay, since the fluorescence of adrenaline is four times greater than that of noradrenaline

The B 3 readings for 0 02 ml extract of 47 cases of essential hypertensive vascular disease ranged from two to 44, only five of these cases showed a reading above 30

(2) *Rapid screening procedure for phæochromocytoma* Lund's procedure (Lund 1949b) for gross isolation of catechol amines from plasma was found to be useful as a rapid preliminary step in the fluorometric screening of urines The differentiation between phæochromocytomas and essential hypertensives however is not as clear cut with this procedure as was found true in the longer method, in cases of a positive result procedure 1c (above) is used as a check

Lund has stated that he has used a similar procedure for

chromatograms (James 1948, Goldenberg *et al*, 1949) For details of the method, we refer to Goldenberg and his associates (1954) By applying 0 05 ml of an acid alcohol urine extract to paper, phæochromocytoma with persistent hypertension or of the paroxysmal type can easily be distinguished from essential hypertensive vascular disease The smallest amount of noradrenaline or adrenaline easily demonstrable on paper by the use of our indicator is 1 μg The highest noradrenaline content found in urine extracts from essential hypertensives was 6 μg per ml which makes the amount contained in 0 05 ml (0 3 μg) invisible on paper Urine extracts from phæochromocytoma with persistent hypertension on the other hand, contain in this series, 42–250 μg noradrenaline or 72 μg adrenaline per ml The urine extracts from paroxysmal phæochromocytomas contained from 20 μg noradrenaline or adrenaline, up to 100 μg adrenaline or noradrenaline per ml Of the 16 cases of phæochromocytoma studied by us fifteen were positive by paper chromatography, in one case the extracts gave unsatisfactory chromatograms—this despite the fact that the noradrenaline content per ml was above the threshold of the method

(1c) Lund has studied the quantitation of adrenaline by photofluorometry (Lund 1949*b*) In our studies of total fluorescence of a urine extract, we have essentially used his adrenaline procedure for developing the fluorescence, as follows oxidation with MnO at *p*H 6 is followed by treatment with a sodium hydroxide ascorbic acid solution This fluorescence is compared with that obtained without ascorbic acid In the latter case the fluorescence of adrenaline disappears within a few minutes while that of noradrenaline disappears in twenty five minutes With the ascorbic acid added to the sodium hydroxide the fluorescence is stable It is this property which permits differentiation of adrenaline and noradrenaline from most other substances in the extracts which exhibit fluorescence in alkaline media (For details of the method, see Goldenberg *et al*, 1954) We have not been able to repeat Lund s (1950) method for quantitation

of mixtures of noradrenaline and adrenaline by differential oxidation at pH 3 and pH 6 5

In our experience the differentiation of phæochromocytoma from essential hypertensive vascular disease can best be achieved by this type of photofluorometric evaluation of the urine extracts obtained by adsorption on precipitated aluminium hydroxide This method does not permit a separate quantitation of adrenaline and noradrenaline in the urine extracts, but is limited to obtaining a total fluorescence value at pH 6 0 using a Coleman 12 B photofluorometer and PC 2 secondary and B1 B2 and B3 primary filters The highest specific readings for adrenaline and noradrenaline are obtained with the B 3 filter, which passes part of the 436 mμ and all of the 405 mμ line B 1 readings (this filter passes the 365 mμ line) and B 2 readings (this filter passes the 436 mμ line) are necessary to screen out non specific fluorescence in the urine extracts In phæochromocytoma B 3 readings are always higher than those of B 1 or B 2 and the ratio B 1 B 2 lies between 0 7 and 2 0, which represents the ratios for adrenaline and noradrenaline respectively

The B 3 readings for 0 02 ml urine extract (100 1) ranged from 138 to 1604 in our 16 cases of phæochromocytoma These readings do not parallel the noradrenaline equivalent per ml values obtained by bioassay since the fluorescence of adrenaline is four times greater than that of noradrenaline

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Lund has stated that he has used a similar procedure for

the quantitation of adrenaline and noradrenaline in plasmas and urines of phæochromocytoma patients (Lund, 1952) We have not been able to confirm these quantitative studies one of the reasons being that the total fluorescence obtained is due only partly to the presence of adrenaline and noradrenaline (For details of our procedure determination of the total fluorescence of acetic acid eluates from aluminium oxide columns previously treated with hydrolysed urine, see *Goldenberg et al*, 1954)

Urine specimens from seven cases of phæochromocytoma were examined using this method The B 3 readings calculated for 2 ml urine were found to range from 270 to 620

There is no doubt that this adsorption is less specific than the long method (1 and 1c) In contrast to the long method (1c) which gave 44 as the highest B 3 reading in the group of essential hypertension (for 0 02 ml urine extract), with a B 1 B 2 ratio of 8 0, the B 3 readings with the short method of the same group ranged as high as 110 considering only B 3 readings which were higher than B 1 or B 2 and showed a B 1 B 2 ratio between 0 7 and 2 0 A single case of essential hypertension in a child showed a reading of

B 1	B 2	B 3	B 1/B 2
75	50	205	1 5

The same urine specimen gave readings of

B 1	B 2	B 3
20	9	20

using the long method (1c)

In so far as the rapid screening procedure is concerned this case is considered a false positive We therefore recommend that any urine sample which gives B 3 readings greater than 110 and has a B 1 B 2 ratio of less than 2 has to be tested by the long procedure (1c) Here is a rough screening test suitable for mass screening of hypertensives, which, in case

of a positive result has to be checked by the more specific method

3 *Pharmacological Tests* An evaluation of the results of pharmacological testing in our group of 16 cases of phæo chromocytoma shows that false negative responses were rarely encountered Out of 10 tumour cases tested with Benzodioxane (Goldenberg Snyder and Aranow 1947) one showed a false negative response Out of six cases tested with Regitine (Emlet Grimson Bell and Orgain 1951) there was one false negative of eight cases tested with histamine one was false negative

It is noteworthy that the only false negative response to Benzodioxane in this group was found in a case who also exhibited a false negative response to Regitine This patient also showed hypertension outlasting the removal of the tumour previously described by us in cases of phæochromo cytoma with false negative response to adrenergic blocking agents (Goldenberg *et al* 1950) The probable mechanism a non humoral phase of *hypertension* in phæochromo cytoma

The incidence of false positive pharmacological response in 91 cases of essential hypertensive vascular disease is listed below

Benzodioxane test repeated false positive responses to Benzodioxane and Regitine were obtained in one case, who showed negative urinary excretion and proved negative on exploration A second case showed a false positive response to Benzodioxane only while the patient was tested for the first time The Benzodioxane test was twice negative on repeat and so was the urinary catechol amine excretion

The incidence of false positive Regitine responses in this group is much higher eight cases of essential hypertension including the above mentioned case and a case of uræmia not included in this figure

These findings necessitate a Benzodioxane test or urinary excretion studies in all cases found to exhibit a positive response to Regitine

Summary

The chemical screening methods suggested permit an easy differentiation of phaeochromocytoma with persistent hypertension from essential hypertensive vascular disease These methods also permit the diagnosis of phaeochromocytoma with paroxysmal hypertension with or without paroxysms during the period of urine collection

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DISCUSSION

VON EULER I have little to add to Dr Goldenberg's remarks I should like to congratulate him on his results and I think actually he has gone further than we have We are still sticking to the rather cumbersome and time consuming bioassay of adsorption and the only advantage I can see is that with the present method the cat's blood pressure and the chicken rectal caecum in the Gaddum preparation we

get a somewhat better separation than seems to be implied by Dr Goldenberg s method There is also a quite striking agreement between the proportion of adrenaline and noradrenaline in the urine and in the excised tumours which I think is most interesting because from the urinary analysis one can get an idea of the composition of the tumour I think it would be a great advantage to use the screening method for these cases which would take less time Recently we have tried using the adsorption on aluminium oxide which seems to be a fairly simple thing just adding the aluminium oxide to the hydrolysed urine filtering it after adjustment to pH 8 5 and then eluting it with 0 2 0 4 N sulphuric acid We get a filtrate which after neutralization can be tested directly on the cat s blood pressure and also on the chicken rectal caecum which saves a lot of time especially when there is a series of samples But I admit it would be better to have a photochemical method which does not involve bioassay especially in hospital laboratories

GOLDENBERG I would rather use the direct method which is technically feasible since the rapid screening procedure takes only about one hour Any pharmacological response shows a certain degree of variability If you have worked long enough in this field you can evaluate pharmacological tests reasonably well This is not equally true for the practising physician who does an occasional test This is one of the reasons why a direct method seems preferable

VON EULER May I refer very briefly to our last case of phaeochromocytoma It was diagnosed as essential hypertension at the neurological clinic and the Regitine and Benzodioxane tests were both negative The patient was considered for a sympathectomy operation and just as a matter of routine they sent down a sample to our laboratory where it was shown to be a case of phaeochromocytoma so they had to operate on the other side so to speak

GOLDENBERG The main objection to the diagnostic use of adrenergic blocking agents is the fact that there is a phase in phaeochromocytoma with persistent hypertension in which the hypertension is not humoral not just comparable to an infusion of adrenaline and (or) noradrenaline but due to a secondary mechanism We have the impression that not more then 10 per cent of the cases will be missed due to this non humoral phase if you test with an adrenergic blocking agent One could therefore also set up the mass screening of hypertensives using Regitine which is preferable to Benzodioxane because of its minor side reactions but which on the other hand gives quite a few false positive results This would make it necessary to screen out the false positives by the use of the urinary method and you would still miss cases like the one reported by Prof von Euler or the single case in our series of sixteen which was pharmacologically negative but easily diagnosed by excretion studies

GOVAERTS Which method would you recommend a clinician to apply to a patient who is supposed to be hypertensive?

GOLDENBERG The rapid screening procedure using the fluorometric evaluation of a hydrolysed urine eluate from an aluminium oxide column

PERERA But should he send you the urine of the patient, or should he do a Benzodioxane test?

GOLDENBERG He should do the chemical part himself, naturally

PEART I think I can confirm Dr Goldenberg's opinion of Regitine Regitine used intravenously as a screening test will produce about 25 per cent of false positives, and you get quite a substantial fall in cases of essential hypertension The trouble with Benzodioxane is that it produces many false negatives but when it is positive it really means something

GOLDENBERG Benzodioxane will give a false negative result if there is not enough circulating noradrenaline and (or) adrenaline present In publications of single false negative cases of phaeochromocytoma the drug has been blamed for the failure of diagnosis It is not the drug but the stage of the disease that matters e g in one of our cases with persistent hypertension there was just not enough circulating noradrenaline present at the time to give a positive response to Benzodioxane or Regitine The classical pharmacological analysis of Benzodioxane by Bovet should be consulted by some of the authors

PEART But there is the observation of Barcroft and I think Swan of giving noradrenaline infusions they failed to abolish the effect of noradrenaline infusions with Benzodioxane I know that in your published case you had in fact done that, but I don't know what the explanation is

GOLDENBERG Dr Aranow and I repeated the same infusion experiments in New York and it worked again We discussed these results with Dr Swan and agreed in only one respect that we did not know why our results differed

PEART Man must be about the only species in which Benzodioxane doesn't reverse the action of adrenaline and abolish the action of noradrenaline—if that were true it would be very surprising

PAGE I think there is another possible way out by using dimethyl piperazinium phosphate to stimulate the production of noradrenaline and catechol amines to get the blood pressure up and then giving Benzodioxane or Regitine which will elicit a precipitous fall

GOLDENBERG This is obviously scientifically an excellent method but I would rather not apply it in patients with phaeochromocytoma Having seen the response to histamine in a case of paroxysmal hypertension I decided to stick to urinary excretion The patient's pressure went up to 300/200 mm Hg shortly thereafter his pulse was unobtainable He was terribly frightened and so was I We were unable to block the large amount of adrenaline poured out following histamine by single doses of Regitine 5 mg intravenously

PEART Many of these patients are on the verge of left ventricular failure and I think that a routine giving of histamine or something like that is rather violent

PAGE Well that's an occasional case only

GOLDENBERG There have been two cases of death

PAGE That's from abuse I think you can control most of them by infusion of Benzodioxane or Regitine I've never been frightened by them

ROSENHEIM Have you ever been frightened by Benzodioxane in someone who hasn t got a phæochromocytoma?

PAGE Yes indeed That s why we have gone over to Regitine

GOLDENBERG I have never had a chance to be frightened by Benzodioxane effects in a case of essential hypertension but such effects have been recorded by others and constitute one of the reasons I am advocating the diagnostic use of urinary catechol amine secretion

PERERA Do you care to tell us something about the excretion values in some of the patients with persistent hypertension after removal of the tumour?

GOLDENBERG Out of nine cases of persistent hypertension due to phæochromocytoma on whom urinary studies were done four cases showed immediate return of the blood pressure to normal four cases died and only one showed hypertension persisting after removal of the tumour This case is being studied now

PLEURA But should he send you the urine of the patient or should he do a Benzodioxane test?

GOLDENBERG He should do the chemical part himself naturally

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pressor amines was 70 µg as the free base It can be seen from Table I that all the patients had an excretion within that limit following operation but two had definite sustained hypertension after long observation (W H and E G)

Table I

TOTAL TWENTY FOUR HOUR URINARY EXCRETION OF PRESSOR AMINES AS FREE BASE (µG)

Patient	Total		B P after operation (Interval in years)
	Before operation	*After operation*	
*W H	—	40	210/140 (3½)
*E G	500	0	?30/140 (1½)
M J	4375	40	165/100 (1)
D K	41?	45	1?0/?0 (1½)

* Patients with most marked hypertension—also in subsequent Tables.

Comparison of Patients with and without Persistent Hypertension

Essential hypertension It is possible that both patients with persistent hypertension may be cases of pre existing so called essential hypertension and that the pheochromocytoma was superimposed Sowry and his colleagues (1953) have shown clearly that there is a familial tendency in the occurrence of essential hypertension and further that blood pressure rises steadily with age in the relatives of patients with hypertension as it does in a random sample of the population Evidence of a familial disposition to hypertension in our patients was not convincing The only sister of one patient (W H) had died from hypertension in childbirth aged ?0 at which age severe essential hypertension is rare His parents pressures were unknown and his brother aged 42 had a normal blood pressure The mother of the other patient (E G) aged 69 had a blood pressure of 170/105 that of the father who died of pneumonia was unknown It may be noted here that the age at operation bore no consistent relation to the persistence of hypertension (Table III) The patients with sustained hypertension were not the oldest

PERSISTENCE OF HYPERTENSION AFTER REMOVAL OF PHÆOCHROMOCYTOMA, WHERE EXCRETION OF ADRENALINE AND NORADRENALINE IS NORMAL

W S PEART

This paper is based on six cases studied at St Mary's Hospital, three of which were previously reported by Barnett and associates (1950) and three reported by Hamilton and associates (1953)

As a result of numerous observations in experimental hypertension, a number of hypotheses have been advanced as to the role of various organs in its causation This arises mainly, of course from the conjunction of the classical work of Goldblatt and his colleagues (1934 1938) on the hypertension produced by renal artery clamping in the dog with clinical observation on hypertension occurring in patients with kidney disease Since then it has been shown that in addition to any renal factor there exist extra renal factors of great importance It is in the effort to link animal experimental observations with human hypertension that the study of cases of phæochromocytoma is important

Persistence of Hypertension when Excretion of Noradrenaline and Adrenaline has Returned to Normal

Table I shows the total twenty four hour urinary excretion of pressor amines as µg of free base In this and all subsequent Tables the patients with the most marked sustained hypertension are shown with an asterisk The method of urinary extraction is based on that of von Euler and Hellner (1951) with subsequent biological assay using the pressor response of the anæsthetized rat (Hamilton *et al* 1953) In 52 patients with hypertension not due to a phæochromocytoma the highest twenty four hour urinary excretion of

during that time was the main factor This view is not supported since three patients (B H R B and M.J) had severe attacks for eight four and two years respectively without persistent hypertension Further evidence on the

Table III

BLOOD PRESSURES

Patient	Age at operation	Blood pressure		
		Before operation		After operation (interval in years)
		Highest	Lowest	
*W H	38	200/140	190/120	210/140 (3½)
*E.G	40	300/190	290/105	250/140 (1½)
B H	35	254/154	190/86	135/80 (3)
R B	49	250/140	290 105	190/100 ([illegible])
M.J	66	260/180	150 85	105/100 (1)
D K.	33	214/105	190/90	190/90 (2½)

severity of the attacks is illustrated in Table III which shows that all the patients except one (D K) had comparable rises of blood pressure during attacks

Size of tumour and rate of urinary excretion of pressor amines A further index of the severity of the condition might have been sought in the size of tumour and the rate of urinary excretion of noradrenaline and adrenaline since these amines might cause a secondary hypertension in other ways than by their effects as pressor substances Mere size of tumour had no influence on the persistence of hypertension since the two relevant patients (W H and E G) had the smallest tumours in our series and the amine content of the one examined (E G) was as low or lower than most of the others (Table IV) The ratio of noradrenaline to adrenaline in the tumours is of no significance either since one of these patients (E G) had a tumour with high noradrenaline content whereas cases 11 and 6 from the series reported by Goldenberg and associates (1950) had a high adrenaline content and showed persistent hypertension after operation

It is therefore suggested that the previous presence of a phaeochromocytoma may be responsible for the persistent hypertension, though this conclusion can only be provisional on such limited data

Length of history It might be assumed that those patients who had the longest history of symptoms would be those who subsequently had maintained hypertension In none of our cases was there any evidence as to the total length of time for which the blood pressure had been raised In the hope that cardiac hypertrophy, as shown radiologically, or by forceful movement of the precordium between attacks might indicate hypertension of longer standing this has been separately considered

No great weight can be placed on the absence of clinical or radiological evidence of cardiac hypertrophy It is not known whether sustained hypertension ever precedes the

Table II

LENGTH OF HISTORY

Patient	*Duration of symptoms (years)*	*Cardiac enlargement*
*W H	2½	+
*E C	5	−
B H	8	−
R B	4	−
M J	2	+
D K	½	−

onset of the typical acute attacks in this condition The findings shown in Table II do not indicate that a long history necessarily leads to persistent hypertension since those with persistent hypertension did not have a longer history than those without Further one case with such persistence (E G) had no cardiac hypertrophy yet marked hypertrophy was present in one patient (M J) without persistence

Severity of symptoms It could be argued that mere length of history is not of importance but that severity of symptoms

W H and B H and in the spleen of case R B In this very limited search no fibrinoid necro es were discovered in the patients with persistent hypertension Papillœdema retinal hæmorrhages and soft exudates were present in both the patients who had sustained hypertension (W H and E G) while retinal hæmorrhages and exudates were seen in two others (R B and M J) (Table V) In all four of these

Table V
RETINAL DISEASE

Patient	Papillœdema	R t nal hæmorrhages and exudat
*W H	+	+
*E.G	+	+
B H	−	−
R.B	−	+
M.J	−	+
D K	−	−

patients the blood urea urea concentration test and intra venous pyelogram were normal but of the two patients with sustained hypertension one (E G) had protein red cells and granular casts in the urine in the other (W H) they were absent Of the other patients two (R B and M J) had a trace of proteinuria

These crude tests of kidney function do not help in deciding whether damage to the kidney is playing any part in the sustained hypertension of either of these patients (W H and E G) since one (E G) had evidence of such damage while the other (W H) had none These two patients were the only ones with clinically malignant hypertension and in particular differed from the rest by the presence of papill œdema However it cannot be claimed that the presence of malignant hypertension necessarily causes sustained hyper tension since I have records of a case of Dr Prunty s at St Thomas s Hospital a girl aged 16 with papillœdema and gross proteinuria and a high excretion of noradrenaline in

The rate of excretion of adrenaline and noradrenaline in the urine was not higher before operation in those patients with persistent hypertension than in those without (Table I) I have observed that the rate of excretion is related as might be expected, to the number and severity of attacks during the period of urine collection, and as previously discussed, the

Table IV

TUMOUR SIZE AND AMINE CONTENT

Patient	*Wt of tumour g*	*Amine content mg/g wet wt*	*Ratio Nor/Adr*
*W H	45	—	—
*E G	46	2 0	9
B H	200	—	—
R B	1100	10 3	33 3
M J	50	1 6	4 0
D K	67	2 4	1 9
11	39 5	0 [illegible]	0 2[illegible]
6	55	0 [illegible]	0 16

*Cases 11 and 6 are from Goldenberg *et al* (19[illegible]0)

importance of this frequency is of uncertain value The patient with the highest excretion (M J) did not have sustained hypertension

Severity of signs Since the duration and severity of symptoms does not throw any light on the persistent hypertension, variation in the response of the individual vascular system might be shown by the severity of the pathological change induced *The incidence of malignant hypertension* can be used as a criterion of this response The only sure evidence of malignant hypertension is the presence of fibrinoid arteriolar necrosis in various organs Clinically the presence in the eye of papillœdema with retinal hæmorrhages and soft exudates is accepted as diagnostic This may be associated with renal damage shown by protein, red cells and granular casts in the urine The presence of fibrinoid necroses was looked for in all the tumours, in renal biopsies from cases

the blood pressure in certain cases of phæochromocytoma was because a secondary mechanism was sustaining the blood pressure and that very little adrenaline or noradrenaline was then circulating In both our cases with sustained hypertension the piperoxane test was negative yet positive in one case

Table VII

THE EFFECTS OF INFUSIONS OF PIPEROXANE

Case	Dose (mg)	B.P mm Hg	
		Before injection (mean)	*After injection (maximal effect)*
*W H	15	185/125	2[illegible]5/130
*E G	8	2[illegible]4/140	250/160
M J	20	200/120	170/70

whose pressure dropped after operation (Table VII) This superficially supports this idea but more direct evidence is needed

Hypotension after removal of the tumour It is of interest to note that the marked drop in blood pressure which occurs within minutes of removing the tumour in most cases was present in both the patients with subsequent hypertension Whatever the mechanism of this fall and it is similar to that which occurs on cessation of noradrenaline infusions in rabbits (Blacket *et al* 1950) and in man (personal observation) it is capable of overcoming the mechanisms causing sustained hypertension unlike piperoxane

Other Reports of Sustained Hypertension Following Removal of Phæochromocytoma

The fullest report of sustained hypertension was that of Goldenberg and associates (1950) and of three cases with a long follow up two had shown reversal of a previously positive piperoxane test and so it could be assumed that no functioning tumour tissue was present

the urine, who had a normal blood pressure nine months after operation Malignant hypertension is quite common in recent case reports, but the only indication and that equivocal, of the possible occurrence of fibrinoid arteriolar necrosis is contained in a paper by Platt and Davson (1950) The retinal changes in both the cases with sustained hypertension soon reverted towards normal

Pattern of the circulation *Blood flow* Investigation of blood flows in these patients has been limited to that through the hand The heat elimination from the hand, made maximal by rapidly heating the body, is a measure of the rate of hand blood flow after release of vasomotor tone from its vessels (Pickering, 1936) It was measured by following the temperature changes of water in a calorimeter in which the hand was immersed, and Barnett and his co workers (1950) have shown that patients with phæochromocytoma have a low hand flow We have confirmed this in two out of the three subsequent patients (Hamilton *et al*, 1953) (Table VI)

Table VI
MAXIMUM HEAT ELIMINATION

Patient	Calories per 100 ml of hand per min	
	Before operation	*After operation*
*W H	3	90
*F G	34	86
B H	24	76
M J	87	80
D K	93	116

After operation the blood flow was normal in all the patients and the patients with persistent hypertension then behaved like other cases of essential hypertension who have normal hand flows The lowest level I have found for the maximal heat elimination in a normal subject was 60 calories/100 ml hand/minute

Response to piperoxane Goldenberg and Aranow (1950) suggested that the reason why piperoxane failed to depress

the blood pressure in certain cases of phæochromocytoma was because a secondary mechanism was sustaining the blood pressure and that very little adrenaline or noradrenaline was then circulating In both our cases with sustained hypertension the piperoxane test was negative yet positive in one case

Table VII

THE EFFECTS OF INFUSIONS OF PIPEROXANE

Case	Dose (mg)	B.P mm Hg	
		Before injection (mean)	After injection (maximal effect)
*W H.	15	18[illegible]/12[illegible]	225/130
*E C	8	[illegible]4/140	250/160
M.J	20	200/170	1[illegible]0/70

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The fullest report of sustained hypertension was that of Goldenberg and associates (1950) and of three cases with a long follow up two had shown reversal of a previously positive piperoxane test, and so it could be assumed that no functioning tumour tissue was present

Discussion

It must be first stated that no conclusions can be safely drawn from such a small series From the results given, the patients with persistent hypertension cannot be differentiated readily from the other patients, but it can be stated that neither malignant hypertension nor any detected form of renal damage is essential for this persistence There is then no evidence to point to either a renal or non renal cause for this persistence but merely the strong suggestion that phæochromocytoma is responsible for a secondary hyper tension

Links Between Experimental Hypertension and Human Hypertension

From the work of Pickering (1945), Grollman and his colleagues (1951) Wilson and Byrom (1941) and Floyer (1951) it seems clear that in addition to any possible renal mechanism there are extra renal factors capable of raising the blood pressure The only one of these which has been at all extensively investigated is section of the carotid sinus nerves (Heymans *et al* 1953) Most attention has been con centrated on the observation that the hypertension produced in animals may persist when what is taken to be its primary cause the kidney is removed Grollman and co workers (1951) in the bilaterally nephrectomized dog have shown most clearly the presence of extra renal factors in experi mental hypertension

The importance of this work in relation to human hyper tension is that it may enable us to understand some of the reasons for sustained hypertension when what is taken to be its primary cause is removed One of the first difficulties which occurs is the absolute proof of a primary cause of hyper tension in man and this before renal and non renal factors can be discussed at all This is where the study of patients with phæochromocytoma is valuable, since if it is accepted that they cause a secondary hypertension, this may be related

to secondary renal changes or to extra renal factors So far we have not enough evidence in any direction

Other Possible Causes of Secondary Hypertension

Other primary causes of hypertension which lead to a secondary hypertension have been little studied from this aspect in man but there is some information about them

Unilateral pyelonephritis The views on this condition extend from those of Goldring and Chasis (1944) who do not believe that hypertension is especially linked with pyelo nephritis to those of various authors who have reported a fall in blood pressure on removal of an affected kidney The latter view has been supported by Pickering and Heptinstall (1953) who gave as examples four patients who had a marked fall in blood pressure after removal of a pyelonephritic kidney, where there was evidence that the other kidney was not similarly affected This definitely points to a renal mechanism for hypertension in these cases but in some cases of apparently unilateral disease the blood pressure does not drop on removal of the kidney These authors point out that this may be due to undetected pyelonephritis in the opposite kidney but this requires pathological proof in all cases It is not possible to invoke by analogy the experiments of Wilson and Byrom (1941) who claimed that in rats made hypertensive by clamping one renal artery the persistence of the hypertension after removing the clamped kidney was related to arteriolar changes in the opposite kidney

Coarctation of the aorta Here is what seems to be a cause of hypertension ideal for study since the duration of its existence is always known though the length of time for which hypertension has existed may not be known From the point of view of study of persistent hypertension the complete removal of the stricture must be accomplished Unless there is evidence that the blood flow through the aorta has been returned to normal levels the reported cases of sustained hypertension cannot be held to support the hypo thesis of a secondary hypertension The largest series reported

was that of Gross (1950), who believes that many failures to relieve the hypertension are due to the failure to remove the stricture completely Out of 80 patients operated on, 79 of whom had resection of stricture with end to end anastomosis or graft, eight had only partial relief, but unfortunately he does not give pre and post operative blood pressure readings It seems that the incidence of post operative hypertension, if the operation is completely efficient, is very low, and from the literature it is not possible to say whether it truly exists

Eclampsia and pre eclampsia These are conditions which may cause marked hypertension and yet, while the primary cause remains unknown it may disappear at the termination of the pregnancy The literature is extremely confusing, since some claim that eclampsia and pre eclampsia frequently lead to persistent hypertension after pregnancy (Dexter and Weiss, 1941) while Barnes and Browne (1945) state that even where sustained hypertension exists the eclampsia merely ' revealed an existing tendency to hyper tension " The indirect evidence in favour of this far reaching claim seems to be fallible and the whole subject throws no light on the mechanisms of primary and secondary hyper tension

Cushing s syndrome due to adrenal tumour Lastly I should like to mention one other cause of hypertension, in which removal of a cortical tumour of the supra renal the supposed primary cause is followed by a return of blood pressure to normal There are a number of case reports in the literature (see Hartman *et al*, 1953 for references) and I have personal experience of one such patient who had a carcinoma of the suprarenal cortex as the culmination of a twelve year history with features of Cushing's syndrome Her blood pressure of 180/120 dropped to 120/80 for six months after operation only to return to its previous high level when metastases developed I have seen no reports of persistent hypertension after removal of such tumours and clearly the tumours must not be associated with hyper plasia of the rest of the cortex—since full removal of the

primary cause must be attained In the follow up of such cases it must be borne in mind that the opposite suprarenal is often atrophied and following operation an Addisonian like state may develop with a blood pressure at a level lower than its ultimate level Therefore the follow up as always must be long

From a consideration of these cases of phæochromocytoma with additional evidence from cases of unilateral pyelo nephritis coarctation eclampsia and Cushing s syndrome, there is only sufficient evidence to point to a definite primary renal factor in the cases with pyelonephritis and there is no evidence from the other cases of renal or non renal factors in a primary or secondary role

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DISCUSSION

PATON There is a pharmacological point here I should not have thought you would expect to get *persistent* hypertension in the cases of phaeochromocytoma because if you infuse adrenaline or noradrenaline experimentally for a long time it is quite difficult to maintain a raised blood pressure Dr Peart is aware of that and has already drawn attention to the fact that you get a big fall when you remove the tumour Now if that is the case and you have a tumour continuously secreting you might easily get a continuous appearance of catechol amines in the urine although the blood pressure rise should not be sustained On the other hand a patient with *paroxysmal* hypertension might easily retain a normal sensitivity to adrenaline This has the implication that if for instance there was a persistent hypertension it might easily be Regitine resistant because the adrenaline or noradrenaline although present would not be doing much

PEART On the point of persistence I don t think you can compare an infusion of noradrenaline or adrenaline as it has been described in the literature with what happens in these patients Infusions of adrenaline or noradrenaline have been carried out at a continuous rate Pickering and his co workers discovered that they could not maintain a hypertension with noradrenaline very successfully the rabbits after two to three days became very sick and then subsequently got a drop in blood pressure when the infusion was stopped What happens when you observe these patients clinically is not like that in many patients it is much more like successive outpourings For example one of our patients had an excretion of 500 μg half an hour after an attack and yet at other times she might have only 500 μg in twenty four hours If you watch the blood pressure charts of some of these patients they are remarkably labile even where the blood pressure is persistently above normal They may shoot up to 300/100 they may drop to 140/100 and then go up again That is not like a continuous infusion I know of no evidence that you cannot maintain separate discrete injections of adrenaline or noradrenaline for long periods and I don t know that you necessarily lose your sensitivity to injections of noradrenaline at short intervals in the same way as you do to continuous infusion

PATON But I thought some of these cases had a sustained raised blood pressure

PEART Yes they do indeed

PATON Are they cases in which the ouput of amines is irregular or intermittent? You might expect to have a rather fixed output

GOLDENBERG Judging from our experience it is easy in man to maintain hypertension at any desired level by a constant noradrenaline infusion This evidence was obtained in cases of phaeochromocytoma after removal of the tumour and in thoracolumbar sympathectomies in whom the blood pressure had to be maintained by constant noradrenaline infusion for a period of days We also observed a case who was decerebrated by prolonged nitrous oxide anaesthesia and was kept alive by continuous noradrenaline infusion for one week Any desired pressure level could be maintained in this case I am afraid that Dr Pickering's results obtained on rabbits are not valid for man In man and in dogs it is possible to maintain hypertension by a constant noradrenaline infusion up to one week

PATON With a constant dose?

GOLDENBERG Yes

PICKERING This infusion of noradrenaline what was the dose what was the rise in pressure and at the end of the week did a given dose produce approximately the same pressure as it had on the first day and was there any profound fall of pressure after stopping it?

GOLDENBERG Yes the fall was quite profound—the patient died It had been obvious that this patient could not survive but he was kept going on artificial respiration and a fairly constant noradrenaline infusion I do not recall the exact figures but our anaesthesia department never infused more than 40µg per minute except in cases of phaeochromocytoma You will find the same true for thoracolumbar sympathectomy if you have to maintain the blood pressure in such a case for a few days you can easily do it with a constant noradrenaline infusion

PICKERING I should feel happier if I knew the value of the arterial pressure after you discontinued the infusion

GOLDENBERG It dropped to zero when we discontinued this infusion It drops to zero if you discontinue the post operative noradrenaline infusion in cases of phaeochromocytoma who have shown persistent hypertension prior to the removal of the tumour

PICKERING In the case of the decerebrate man you cannot assume that the blood pressure value is due to the noradrenaline unless you have a control period afterwards in which you can show that the pressure is roughly the same as it was before you started the infusion

GOLDENBERG This point was definitely established stopping the noradrenaline infusion caused a *precipitous* drop of the blood pressure to zero levels

PAGE Surely the difference is that you were *dealing* with a decerebrate preparation In other *words* it was like a cord-destroyed dog in that if you infuse noradrenaline you don't get the depressor effect—the blood pressure goes up and stays up for days if the cord dog stays in good shape These dogs *exhibit denervation* super sensitivity so that smaller amounts of noradrenaline will produce much greater effects Nor do you see the fall in pressure when you withdraw the drug from a cord dog

GOLDENBERG If you take a phæochromocytoma with an excretion of 2 7 mg noradrenaline in twenty four hours and calculate the approximate rate of secretion by the tumour the calculated amounts are sufficient to maintain persistent hypertension for this length of time It is hard to explain the profound post operative drop of the blood pressure in these cases unless you assume that the tumour had been secreting steadily and maintaining the elevated blood pressure level The same is true for the fact that in eight out of nine cases of phæochromocytoma with persistent hypertension (in this group) adrenergic blocking agents abolished the hypertension This again supports the idea that the persistent hypertension in these cases was due to circulating adrenaline and (or) noradrenaline and is therefore similar to the effects of a continuous noradrenaline infusion

It is the same story as with the hæmodynamic effects of noradrenaline and adrenaline The response differs so much from species to species that I would hesitate to transfer to man the results obtained in rabbits

PLATT May I briefly refer to our case that Dr Peart mentioned in his paper As far as I remember the man behaved clinically exactly like a malignant hypertension and that was in fact the diagnosis which was made His renal function was already very bad when he was admitted to hospital that is he had nitrogen retention He died very soon afterwards Bilateral phæochromocytomata were found at autopsy which had not been suspected during life and he did have arterial necroses and all the appearances of malignant hypertension in his tissues

PICKERING I wonder if your experience is anything like mine We have now had eight cases in which one kidney has been removed for unilateral pyelonephritis and of these four have had very conspicuous falls of blood pressure and four have not All the four that have not have either had papillœdema or have had arteriolar necroses found in their kidneys Of those who have had conspicuous falls one had arteriolar necroses and papillœdema and not one has returned to what would be ordinarily called a normal arterial pressure that is to say the diastolic runs at about 100 or over

PLATT No I wouldn t like to classify mine most of which are failures rather than successes But I would say that the greatest success I ever saw was in a child who had papillœdema and gross retinopathy which completely cleared and was normal five years later

MCMICHAEL Dr Peart put his finger on another very important point when he mentioned coarctation It seems now from experience of this operation that the pressure stays high in about half the cases though down a little (Hallenbeck *et al Surg Gynec Obstet* 92 75) This seems to put it in the class of cases in which you have some factor mechanical perhaps which sets up the hypertension and once raised removal of the original cause will not necessarily lead to cure Many of these patients do get symptomatic relief especially of the feeling of tension and fullness in the head I had one patient whose pressure was first found to be just a little bit up when aged nineteen When aged twenty six he was rejected for life insurance because his pressure was

still higher and it continued to rise until the age of thirty when his coarctation was resected He is immensely better since the operation but he still has hypertension. This is an important group to study

Now I have a practical question It looks as though it is only the paroxysmal hypertensions in your phaeochromocytomas that really come back to normal If so is there likely to be any practical therapeutic gain from the complicated study necessary to establish the diagnosis in persistent hypertensions due to phaeochromocytoma?

GOLDENBERG I would not think that that is generally valid We have seen quite a few cases of phaeochromocytoma with persistent hypertension In our first series published in 1950 out of twelve cases five showed immediate return to normal blood pressure seven showed hypertension outlasting the removal of the tumour In one case it lasted only for three days in another for four weeks The latter case probably constitutes the best argument against the idea that in these cases a rare disease phaeochromocytoma could co-exist with a rather frequent disease essential hypertension It was a girl of about twelve who had two tumours removed and kept a well pronounced hypertension for about four weeks then returning to normal she has had normal blood pressure values since i e for eight years In four cases the hypertension outlasted the removal of the tumour for months and only one case has had hypertension for ten years after removal of the tumour By the way in two of these cases of transient hypertension after removal of the tumour renal blood flow was normal at the time when hypertension was still maintained

BEIN From the pharmacological point of view one is not forced to assume in these cases where the excretion of adrenaline and noradrenaline is high without paroxysmal reactions that there is a substance present in the body which could counteract adrenaline or noradrenaline One could also imagine that adrenaline and noradrenaline are only sensitizers to another substance or stimulus whose nature is not yet known and which produces the rises in blood pressure In experiments with histamine in the spinal cat we know for example that the blood pressure rise is dependent on the concentration of adrenaline and noradrenaline present in the circulating blood because we get the biphasic histamine reaction in the spinal cat also after bilateral adrenalectomy when we infuse adrenaline and noradrenaline And this histamine effect is greater if a higher concentration of these two sympathetic pressor amines is infused (*Arch exp Path Pharmak* 1953 **219** 273)

GOLDENBERG I cannot concur with this hypothesis First I would not like to be recorded as having definitely stated that there is an adrenergic blocking agent present in the body I just wondered how one could explain the findings that a patient harbouring a phaeochromocytoma who does not show a blood pressure elevation for twenty four hours may excrete 600g of noradrenaline in his urine

PEART Certainly these patients maintain for quite a while after operation their resistance to the pressor effects of noradrenaline Quite often when their blood pressure drops you have to infuse extremely

large quantities 10 mg in a litre of saline and run that in fast to bring the blood pressure up They are very resistant

GOLDENBERG This is one of the reasons why I am hesitant about the therapeutic use of adrenergic blocking agents in phaeochromocytoma It is hard enough to restore the patient's blood pressure after removal of the tumour even if you have not applied adrenergic block We had to infuse up to 100 μg per minute to maintain a good pressure level

GOVAERTS Has anyone measured the reactivity to adrenaline in such patients before and after operation?

GOLDENBERG The sensitivity to adrenaline and to noradrenaline is extremely low while the tumour is still in You would expect this because the concentration action curve is a hyperbola and on the upper limb of the hyperbola you have to add very large amounts of adrenaline to get any kind of pressure increase This condition seems to persist after removal of the tumour for a certain length of time which shows that other factors are contributing in decreasing the sensitivity to infused adrenaline and (or) noradrenaline in cases of phaeochromocytoma prior to and shortly after removal of the tumour

GOVAERTS Then you didn't find any difference in pressure before and after operation?

GOLDENBERG We did find differences in blood pressure and sensitivity before and after operation The blood pressure drop is immediate the return to normal sensitivity takes days

PEART Yes I think that is absolutely true An observation made by Barnett and his colleagues on one of the other cases was that one patient who had a normal blood pressure at the time of measuring the maximum heat elimination had a maximum heat elimination that was low Now this is very difficult to understand—is it possible that adrenaline and noradrenaline circulating all the time in this patient without hypertension could be constricting the blood vessels of the skin of the hand without producing a rise of blood pressure? That raises the quantitative problem is it possible to infuse noradrenaline or adrenaline to reduce the skin flow through the hand without raising the blood pressure? Otherwise you may have to invoke some sort of depressor or dilator reflexes to explain why the blood pressure is down

PICKERING Another possible explanation is that if there were only two estimations of blood pressure before the heat elimination and after it it is just possible that it might be high during the actual heat elimination measurement

GOLDENBERG The fact remains that a high noradrenaline excretion has been observed by us in paroxysmal cases without any paroxysms during the time of urine collection

PICKERING Well I take it that a paroxysm is one where the excretion passes a certain value to give symptoms isn't it? There may be fluctuations of excretion which could perhaps be recognised if you had a record of the excretion in great detail

PEART We had as a patient a rather introspective doctor who observed during a twenty four hour period very minor attacks which other people might not have observed The question of what you define

as a paroxysm becomes rather important because they can well have minor attacks which might pass notice and they may well excrete large quantities during that time

PICKERING Whilst we have this great concentration of talent available I d like to ask two elementary questions Firstly is there any relationship between the amount of adrenaline and noradrenaline excreted in the urine and the amount infused or introduced into the body? And secondly have we got agreement about what is the rate of excretion in essential hypertension?

VON EULER We have made a few experiments on that and in the case of noradrenaline the excretion appears to be between 1 5 and 3 3 per cent of the total amount injected per unit of time I know that Dr Goldenberg has found sometimes a little more than that

GOLDENBERG 4 per cent using the twenty four hour excretion

VON EULER Quite recently we have repeated that with adrenaline and the figures are slightly less between 0 4 and 1 7 per cent of the total amount infused

PATON Is the variability such that you can t say what are significant changes in excretion or not? The figures you have given suggest a three fold range of variation in the excretion

VON EULER Yes that s what I should say we found These observations were all on normal subjects medical students under control conditions

PATON People might tend to think that if the excretion went down from 1500 to 500 μg /day then the paroxysm had passed off whereas in fact it might be just some change in the way the material was handled by the body

VON EULER That is quite correct and I think it is a very pertinent remark because even in subjects in whom we have repeated these experiments the rate of excretion has varied quite considerably So I don t think one can place too much reliance on a difference of say from 1000–2000 μg per day

GOLDENBERG The percentage of excreted noradrenaline decreased with increasing *speed of infusion* The percentage varied from 0 3 to 4 per cent in seven subjects the highest was observed with 6900 μg noradrenaline infused within four hours

VON EULER We had one case of phaeochromocytoma in which Dr Lund of Copenhagen measured the catechol amines of the blood with his method He found that the urinary excretion of noradrenaline (and we measured it too) was about 1 8 μg per min at a blood concentration of 3 6 μg per cent

PICKERING But what about the rate of excretion in essential hypertension?

VON EULER Perhaps I can show you a picture of that tomorrow

EXPERIMENTAL STUDIES ON THE PATHOGENESIS AND NATURE OF HYPERTENSIVE CARDIOVASCULAR DISEASE

ARTHUR GROLLMAN

That the kidney plays an essential role in the pathogenesis of most cases of clinical as well as of experimental hypertension is now generally accepted, as is also the view that this process is mediated by some humoral mechanism. The nature of this humoral mechanism is however, still a matter of discussion, nor is there any unanimity of opinion as to the factors which bring about the defect in renal function which is responsible for hypertensive cardiovascular disease. Some also refuse to accept hypertension as it is observed in the human as being the counterpart of the disorder as it is induced in the experimental animal. This is particularly true of the so called "essential" or "benign" type of human hypertension in which the kidney as observed by the usual methods of histological examination appears to be intact morphologically. The role of the nervous system and of the endocrine organs, particularly the adrenal, in the evolution of hypertension is also the subject of much speculation.

The experimental data to be presented offer evidence which renders untenable many concepts which have dominated this field of investigation. The results however suggest alternative explanations which permit one to harmonize many apparent discrepancies and allow one to account adequately for the clinical as well as the experimentally observed facts on the basis of a relatively simple hypothesis.

The Limitation of the Renal Pressor Mechanism as the Mediator of Hypertension

The earlier workers in this field followed an observation of Tigerstedt and von Bergmann in the last century and

assumed that the elevation in blood pressure induced by renal disturbances was mediated by the elaboration of a pressor agent (or its precursor) by the kidney. This concept still dominates the text books and much of the literature in this field, although several crucial experiments fail to substantiate it, e.g. the failure to demonstrate such a pressor agent in chronic hypertension both in the experimental animal as well as in the human disease. It is only under special conditions that such a pressor agent is found, e.g. in eclampsia, after severe hæmorrhage, after ligation of the ureter, after infarction of the kidney or after complete or almost complete occlusion of the renal artery. This form of hypertension probably has its counterpart in those rare instances in the human in which infarction of the kidney results in the very rapid development of a malignant form of hypertension which can be relieved by removal of the affected kidney. When induced in the experimental animal by complete or almost complete occlusion of the renal artery or by ligation of the ureter, the rise in blood pressure occurs promptly but subsides as the kidney atrophies (cf. Fig. 1).

Hypertension Induced by Nephrectomy

The type of hypertension just described differs in many respects from that observed in the chronic hypertension seen in the human and induced experimentally by a variety of procedures involving injury to the kidney. The latter form of hypertension, which constitutes the preponderant variety observed clinically, develops slowly, requiring in the experimental animal at least several weeks and in the human many years for its evolution. It is not ameliorated by removal of the defective kidney. In fact, it may be induced in a severe form by nephrectomy, with all the hæmodynamic and pathological features observed in fulminant forms of hypertension as observed in the human.

The objection has been raised that the hypertension induced by nephrectomy is a result of over expansion of the extracellular fluid volume. The invalidity of this objection has

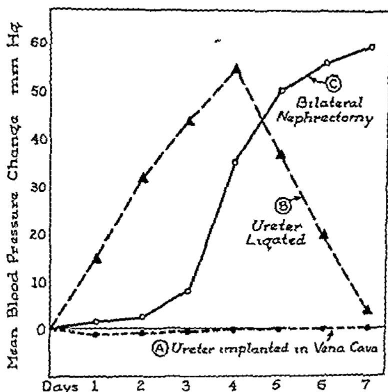


FIG 1. A comparison of the effects of nephrectomy, ligation of the ureter, and implantation of the ureter into the vena cava of the dog. Each curve represents the average of three experiments and shows the changes in blood pressure from the pre operative level induced by the above mentioned operations. In each case the right kidney had been removed several weeks prior to the beginning of the experiment, indicated as Day O on the axis of abscissæ. At this time the left ureter was implanted into the vena cava in the experiments of Curve A (A ●- -●), it was ligated in the experiments of Curves B (▲- -▲) and the left kidney was removed in the experiments of Curve C (O——O). It will be noted that ligation of the ureter resulted in a prompt elevation in blood pressure which, however, gradually returned to normal in the course of a week. Implantation of the ureter into the vena cava induced no change in blood pressure, while removal of the second kidney resulted in a gradual but persistent increase in the arterial blood pressure which remained elevated until the cessation of the experiment. All the animals were fed orally a fat sugar emulsion in water which allowed them 25 ml of water daily (insensible perspiration) and adequate calories to repress their normal rate of endogenous protein catabolism to a minimum.

been demonstrated (Grollman 1953b) and may be inferred from Fig 1 in which the blood pressures are reproduced from a series of bilaterally nephrectomized dogs which were maintained following nephrectomy by the daily injection intravenously of 25 ml of 10 per cent glucose per kg of body weight a volume of fluid less than the volume of the insensible water loss

The Deficiency Theory of the Pathogenesis of Hypertension

The experiments just cited as well as others reviewed elsewhere (Grollman 1946 1947 1953a) support the theory that the kidney normally exerts an incretory as well as its established excretory and metabolic functions *It is the* exclusion of this incretory function which according to this view results in the development of hypertension by such diverse experimental procedures as nephrectomy the partial occlusion of one artery or compression of one kidney with removal of the contralateral kidney or injury of the kidneys by poisons or nutritional deficiencies Hypertensive disease as observed in the human may likewise be attributed to a defective incretory function which may be inherited as a congenital defect in so called familial or 'essential hypertension or may be acquired by a variety of means all of which have in common only the fact that they injure or are accompanied by a defect in function of the kidney The latter group include infections (various forms of the nephritides) such obvious structural defects as congenital lesions hydronephrosis etc and vascular disturbances The last named may accompany a variety of conditions for example nephrosclerosis the disturbances of the renal circulation observed in Cushing s disease disseminated lupus periarteritis etc Despite the apparent lack of any common ætiological factor in all of these conditions one may assume justifiably that they often induce hypertension by their interference with the normal incretory activity of the kidney

The deficiency or incretory theory of renal function

just described allows one to explain in a satisfactory manner many features of hypertensive disease as observed clinically as well as in the experimental animal, and *permits one to* relegate a seemingly unrelated group of conditions to a common underlying defect in the kidney It is necessary, of course, to exclude the relatively few instances of an *increased blood pressure induced by the production of a* pressor agent, by sympathomimetic agents (as in chromaphil cell tumours) by expansion of the extracellular fluid (as by deoxycorticosterone administration and salt), etc These conditions are marked by a rise in arterial blood pressure but not by the other hæmodynamic clinical or pathological features of hypertensive cardiovascular disease Failure to appreciate this differentiation has led to much confusion in the field of experimental hypertension (Grollman, 1953*a*)

Renal Extracts

The most direct evidence for the incretory role of the kidney in the pathogenesis of hypertension would involve the production from kidney tissue of an extract which, administered as a replacement therapy would lower the blood pressure and counteract the other defects observed in hypertension Although claims for the existence of such extracts have been made for some years their elaboration on a large scale has never been successful This may be accounted for by the relatively large amounts of tissue required for preparing effective extracts Many have rightfully looked askance at the claims for the hypotensive effects of such extracts when administered parenterally since many noxious *agents when so administered lower the blood pressure* This objection is entirely valid and the depressor effects of most renal extracts, as well as those prepared from other sources must be attributed to such non specific deleterious effects rather than to the presence of any *physiologically important* agent in such preparations However, the claims for a depressor effect of extracts administered orally to rats cannot be dismissed in the same manner In order to avoid the

contamination of the extract by ammonium sulphate as prepared by the method of Grollman Williams and Harrison (1940) extracts may be prepared by extraction with 70 per cent ethyl alcohol concentration of the resultant extract at 40 C and fractional precipitation with acetone and water It is difficult to attribute the effect of an extract prepared in this way and administered orally to its toxic effects However it must be emphasized that the yield of active material in terms of the amount of kidney used is slight which has militated against performing the obvious experiments required to isolate and study the effects of the active principle

A depressor action similar to that exerted by renal extract is also obtainable from certain oxidized hydrolysates of marine oils (Grollman 1945) Study of these has also been limited by the difficulty in obtaining and preparing active concentrates Preliminary studies suggest an identity of the active agents derived from kidney and oil sources which is considered simply as a fortuitous circumstance

The Fundamental Nature of the Hypertensive Process

Much of the confusion characterizing the present day literature on hypertension is a result of designating any condition accompanied by an elevation in blood pressure as hypertension The limitation of the term hypertension or hypertensive cardiovascular disease to a specific disease entity with certain definable physiological clinical and pathological characteristics allows one to differentiate it as a separate and distinct disorder and avoids the obfuscation otherwise inevitable When the term is thus limited to a specific and definite entity observed widely clinically and its exact counterpart which may be produced experimentally the above mentioned confusion may be dispelled and the established facts harmonized into a relatively simple and logical concept According to this view the elevation in blood pressure represents only one manifestation of a systemic disease involving deviations in many other physiological functions e g in an alteration in the salt and water

metabolism (Laramore and Grollman, 1950), in the relative distribution of the fluid compartments of the body (Grollman and Shapiro, 1953), and probably in many as yet unrecognized alterations from the normal

The widely held assumption that the nervous system is *implicated in the pathogenesis of human hypertension is not* supported by critical observations nor have therapeutic attempts based on this assumption proved satisfactory Likewise the assumption linking adrenal cortical activity with the development of hypertension despite its plausibility, is not supported by experimental fact (Turner and Grollman, 1951)

Summary

The experimental basis for the "renal deficiency theory" of hypertensive cardiovascular disease is outlined According to this view, hypertensive disease as it occurs spontaneously in man or as it is induced in the experimental animal is a result of a defect in an incretory function of the kidney A renal pressor mechanism may be concerned in eliciting an acute rise in blood pressure but this should not be confused with chronic hypertensive disease The adrenals and the *autonomic nervous system are also* considered as providing subsidiary factors which may influence but which play no fundamental role in the pathogenesis of hypertension

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DISCUSSION

PICKERING Can you demonstrate anything in the blood of nephrectomized hypertensive animals? Supposing you take that blood and put it into another nephrectomized animal do you get a response?

GROLLMAN No one cannot demonstrate any pressor agent in the blood of nephrectomized hypertensive animals However one can demonstrate the presence of such an agent in animals in which the ureter has been ligated or in which severe constriction of the renal artery has led to marked ischemia or infarction of the kidney

PICKERING Have you cross transfused them tried to exchange their whole blood volume?

GROLLMAN Yes we have given them large volumes of blood without inducing an elevation in blood pressure This experiment has also been performed on the human in another laboratory The blood of a hyper tensive patient was cross transfused with a normotensive individual with cancer without inducing any rise in blood pressure in the latter

HELMER Dr Shipley and I have removed the kidneys from dogs made hypertensive by renal artery clamping or wrapping the kidneys with silk and found that the elevated pressures were maintained until shortly before death

GROLLMAN How long afterwards?

HELMER The duration of the hypertension before the kidneys were removed was from twelve days to as long as five months My colleague Dr Shipley did an experiment on such a nephrectomized hypertensive animal that is worth mentioning particularly in the light of work reported recently by Wakerlin at the last meeting of the Federation of American Societies for Experimental Biology Wakerlin found that the injection of serums of high anti renin titre intravenously into chronic hypertensive dogs of the Goldblatt type caused a lowering of pressure Dr Shipley performed a similar experiment in a renal hypertensive dog that had been bilaterally nephrectomized In this instance the high anti renin titre serum caused no lowering of pressure This is only one experiment and the value of the work must be judged on this basis At least in this instance there was no pressor agent of renal origin concerned with maintaining the pressure of a nephrectomized hyper tensive dog

GROLLMAN Yes I agree but I am sceptical of the claims that one can effectively treat hypertension by inducing an immunity to renin

HELMER I disagree with you on that. Both Goldblatt and Shipley and I have repeated Wakerlin's work on the reduction of pressure of chronic hypertensive dogs by the injection of kidney extracts with the consequent production of high anti renin titres in the serums of such dogs After the cessation of the kidney extract injections the pressures returned to the previous hypertensive levels with a disappearance of the anti renin in the plasma A second course of kidney extract injections lowered the pressure in a week or so with a production of even higher anti renin titres in the same way as you pep up a toxoid reaction with a booster injection. As far as I am concerned these experiments

demonstrated to me that at least in the Goldblatt type of hypertension there is a positive protein like factor liberated by the kidney that could account for the elevation in pressure

GROLLMAN Might not this be a non specific effect such as would be obtained by injecting any foreign protein?

HELMER No it is not a non specific effect because the titre correlates with the change in pressure

GROLLMAN One would have to conclude from your experiment that the kidney is essential for eliciting the immune reaction I doubt if this view would receive support from the immunologists

HELMER At least the animal with kidneys present had a lowering of pressure

GROLLMAN You are assuming that if the blood pressure is 220 mm of mercury today and if it remains at this level following the removal of the kidney that you have suddenly and instantaneously altered the mechanism responsible for the maintenance of this hypertensive state Such an assumption appears to me to be far fetched and most improbable

HELMER For the time being I support the theory of a positive factor produced by the kidney that elevates blood pressure You support the theory that in hypertension a factor is missing that prevents the rise in pressure Our views may not be so diverse We both agree that the kidney plays a role in the aetiology of hypertension The kidney also is concerned in the regulation of mineral metabolism Regardless of whether hypertension is produced by clamping the renal artery DCA and NaCl administration or by nephrectomy and peritoneal lavage we may be ultimately dealing with some common metabolic change whether it be in electrolyte metabolism or what have you The same reasoning may apply to the discussion of the difference in results obtained when serums of high anti renin titre were administered to hypertensive dogs before and after nephrectomy Before nephrectomy a humoral factor might be present that could affect metabolic processes regulated by the kidney The neutralization of these factors by the anti serums could lower pressure After nephrectomy the anti serum would be without effect since all kidney regulation is removed So perhaps we have not altered the support of the elevated pressure but have only changed the manner in which it is applied

BRAUN MENENDEZ I should like to ask Dr Grollman if he has measured the extracellular fluid volume in the dogs in which he says that there is no increase in weight?

GROLLMAN In the case of the short term experiment no artificial measure was used to prolong life In these cases any increase in extracellular volume which is observed is small and represents the slight expansion of extracellular fluid volume characteristic of hypertension as seen in both man and the experimental animal as has been reported recently (*J clin Invest* 1953 32 312)

BRAUN MENENDEZ If it comes from extraneous sources the hypertension is more manifest You can get hypertension rapidly by injecting sodium chloride solution for instance

GROLLMAN As I have reported in a paper which is now in press (*Amer J Physiol* 1953 173 364) it is necessary to inject a considerable amount of fluid rapidly in order to maintain an increase in the blood pressure Although a decrease in extracellular fluid volume causes a rapid decline in blood pressure it is more difficult to increase the blood pressure by expansion of the extracellular fluid volume

BRAUN MENENDEZ I was talking about the nephrectomized dog

GROLLMAN In the nephrectomized dog the elevation in blood pressure which normally occurs will accordingly not be evident if the animal depletes its extracellular fluid volume by vomiting or diarrhoea Repleneshment of this induced deficit will in turn cause a rise in blood pressure to hypertensive levels. Failure to appreciate this phenomenon accounts for the recent claims of Orbison Leonards and co-workers Over expansion of the extracellular fluid volume in the hypertensive nephrectomized dog will actually depress the level of its blood pressure since it induces heart failure

BRAUN MENENDEZ But the blood pressure is already high.

GROLLMAN The initial level of the blood pressure is immaterial Correction of a depleted extracellular fluid will invariably cause a rise in the blood pressure regardless of its original level The blood pressure will rise to the level (normal or hypertensive) characteristic of the animal prior to the depletion of its extracellular fluid volume In the animal with a normal extracellular fluid volume expansion of this by the injection of fluid will result in a further rise in blood pres ure temporarily but may in the hypertensive also cause a decline by inducing heart failure

PICKERING That s with saline?

GROLLMAN Ringer lactate solution which doesn t produce any change in electrolyte composition

PAGE Dr Kolff in our laboratory has approached the problem from the point of view that the hypertension was due to a large increase in extracellular fluid volume and I must say that at first we thought that was true But our results now confirm Dr Grollman s that the increase in extracellular fluid volume is a relatively minor thing as regards its effects on arterial pressure Excessive administration of electrolyte solutions almost certainly augments the hypertension I would like to ask Dr Grollman whether he thinks an increase in extracellular fluid volume has anything to do with or is a characteristic of essential hypertension whether he thinks the figures are sufficiently impressive to make one believe it a characteristic lesion

GROLLMAN The observed differences are statistically valid both in patients and in the experimental animal

PAGE That doesn t mean very much as regards the mechanisms of the disease

GROLLMAN I think they are significant as measured in the dog by means of mannitol in the human by inulin and in both species by radioactive sulphate The differences are not large but they are definite Why not accept them I am not implying that the observed expansion in the extracellular fluid volume is of fundamental significance or that

it plays a role in the mechanism of the rise in blood pressure I am merely citing a demonstrable fact

WILSON You may say that it is not clamping of the renal artery which produces the hypertension but removal of the opposite kidney But we have always worked on animals which developed hypertension after clamping the renal artery without removal of the other kidney Why does not this normal kidney tissue prevent the blood pressure from rising?

PLATT May I supplement that? How do you explain the few authentic cases of human unilateral renal disease with hypertension in whom nephrectomy has brought the blood pressure to normal?

GROLLMAN We can explain these phenomena on the assumption that both in the rare cases in the human cited by Prof Platt and in the experiments in the rat cited by Prof Wilson infarction of the kidney occurs with the production of a pressor agent (possibly renin or angiotonin) Removal of the affected kidney in these cases results in an alleviation of the hypertension If the kidney is not removed the circulating pressor agent may ultimately cause vascular necrosis in the opposite kidney and thus perpetuate the hypertensive state

Moreover there is a difference in the reaction of different species Dogs rarely have spontaneous renal disease and hence placing a clamp on one kidney results only in a temporary rise in blood pressure when the contralateral kidney is removed the elevation in blood pressure becomes permanent In the rat or rabbit on the other hand in which species renal infection is more common the application of a figure of eight ligature to one kidney or comparable unilateral operations will in a certain proportion of animals result in the development of permanent hypertension

BRAUN MENENDEZ You can put on two clamps you need not remove tissue to get hypertension

GROLLMAN Yes but when you apply clamps to both kidneys you are obviously not dealing with unilateral kidney disease and can assume that you are interfering with normal renal function In the rat and rabbit unilateral operation of the type we perform fails to result in hypertension unless there is pre existing disease of the contralateral kidney (Halpert and Grollman *Proc Soc exp Biol N Y* 1949 71 391)

WILSON I don t think that s true

GROLLMAN I agree that disease of the opposite kidney is not a prerequisite to the development of permanent hypertension following a unilateral operation if one uses the procedure that you have followed In this case infarction of the kidney presumably results in the development of a pressor agent which causes vascular necrosis of the opposite kidney as has been demonstrated by Toussaint (*J n belg path*, 1952 21 465)

FLOYER It is perfectly possible to put a clamp on one renal artery of a rat without causing infarction of the kidney This kidney remains histologically indistinguishable from normal and yet the rat develops chronic hypertension We have observed that initially the opposite kidney is completely normal Lesions certainly develop later on when

the blood pressure rises to high levels but initially you have one histologically normal clamped kidney and one histologically normal opposite kidney nevertheless the animal is hypertensive I agree with your thesis in general and hope to expand it more this afternoon but we need an explanation for this observation

WILSON I think this misconception which Goldblatt originated that the opposite kidney was diseased to start with is most unfortunate The real answer is that it depends entirely on what sort of rat colony you have if you have a healthy rat colony it is just not so

GROLLMAN I am in entire agreement with your viewpoint I believe that we are dealing with two separate phenomena and this has led to confusion In the ca e of low grade renal disease a unilateral operation interferes sufficiently with renal function to induce hypertension When a more drastic operation on the kidney such as the procedure you used results in the liberation of an agent resulting in vascular damage of the other kidney unilateral operation in the absence of pre existing renal disease will induce permanent hypertension In both cases the end result is the same ie interference with normal renal function

WILSON The time of development of the lesion is surely crucial to the arguments whether it is there to start with or whether it develops secondary to the hypertension

FLOYER Dr Grollman supposing in a rat you clamp one kidney and the other remains structurally normal After hypertension has persisted say about three to four weeks (which for a rat is moderately chronic hypertension) you then remove the clamped kidney would you expect the blood pressure to remain up?

GROLLMAN Yes unless you assume that the presence of a clip on one kidney is interfering with the normal function of the contralateral one

FLOYER Yes you have got to make that assumption that the clamped kidney produces a functional change in the opposite kidney without necessarily initiating structural damage Structural damage occurs later after the development of hypertension

GROLLMAN On the other hand if you remove that kidney and find that the blood pressure remains high I would expect that you would be able to demonstrate some morphological change in the remaining kidney

FLOYER You can—I ll expand that this afternoon There seem to be these two phases first of all a temporary functional change in the opposite kidney during which removal of the clamped kidney will bring the blood pressure down and later a permanent structural change when on removal of the clamped kidney the blood pressure remains up

BING Have you tried in your nephrectomized animals to transplant the kidney? I know the life of the transplanted kidney is short but you might transplant one and have it for say six hours and then change and have a new one You might see some results

GROLLMAN That has been done by Dr Sturman at our institution with a resulting decline in the blood pressure He has transplanted a normal kidney to the neck of a nephrectomized hyperten ive dog and noted a drop in blood pressure However I do not consider such experiments convincing

BRAUN MENENDEZ Why don't you think it is good evidence?

GROLLMAN Simply because the shocking effect of an operation and the presence of heterologous tissue might be the cause of the observed decline in blood pressure rather than any true incretory activity

BRAUN MENENDEZ Does it work with other organs? If you grafted a leg for instance or a pancreas and it didn't bring the pressure down—

GROLLMAN No we have not transplanted organs other than the kidney The experiment has been repeated several times with a reduction in blood pressure throughout the life of the transplant which may last *for a week or more*

VON EULER Is there any indication of intoxication of the sympathetic nervous system *in these hypertensive dogs after bilateral nephrectomy?* Is there any alteration in pulse *rate or cardiac output?*

GROLLMAN The cardiac output remains normal despite the elevation in blood pressure Changes in the pulse rate are not significant the venous pressure remains normal There is accordingly no evidence of alteration in the sympathetic nervous system only the hemodynamic changes characteristic of hypertension ensue

VON EULER And what is their response to carotid occlusion?

GROLLMAN I have not investigated that

VON EULER It would be *interesting to see whether that is increased* or changed at all

PAGE I can help to answer that We find that the sympathetic nervous system has no effect You get the same *thing* even in a dog with the spinal cord destroyed—response to carotid occlusion is perfectly normal I had hoped that it was due to the sympathetic nervous system but it isn't

HELMER Your dogs develop hypertension in the absence of adrenals as well as kidneys don't they Dr Grollman?

GROLLMAN Yes following the removal of both the adrenals and the kidneys hypertension still develops (Turner and Grollman 1951 *Amer J Physiol* **167** 462) Such animals *will survive* for one or two months without hormonal therapy

BRAUN MENENDEZ No extra salt?

GROLLMAN No they need be given no excess of salt but *merely* maintained in a normal state of electrolyte and water equilibrium Removal of the kidneys prevents the *loss of electrolyte* and water responsible for inducing acute adrenal insufficiency

HEYMANS I should like to go back to the physiological mechanisms *of the regulation of the blood pressure at normal level In* intact animals the blood pressure is maintained at normal levels by the sino aortic moderator nerves Because in this mechanism *the condition of* the arterial wall where *the receptors* of these moderator nerves are located plays a fundamental role and because in nephrectomized animals hypertension is associated with vascular lesions is it *not* possible that the hypertension in *the nephrectomized* dog might be induced by *a* primary alteration of the arterial wall?

GROLLMAN There is no question as to the involvement of the arterioles the vascular *necrosis* is evident even on gross examination

HEYMANS Does restoration of normal kidney function bring the blood pressure down although extended vascular lesions are present?

GROLLMAN Once the lesions of arteriolo- or of atherosclerosis have been induced and the blood vessels have lost their normal elasticity one would not expect a return to the normotensive state However a reduction in diastolic pressure should occur since the abnormal cause of an increased peripheral resistance has been eliminated In other words we should observe an increased systolic but a normal or decreased diastolic pressure such as is characteristic of the arteriosclerotic process

HEYMANS In neurogenic hypertension induced in dogs after section of the sino-aortic moderator nerves the blood pressure stays at high levels but vascular or kidney lesions do not occur Some dogs have been controlled up to eight years after section of their moderator nerves High blood pressure alone thus does not provoke vascular lesions but it is quite possible that in nephrectomized dogs vascular alterations and lesions are induced which disturb the physiological mechanisms maintaining blood pressure at normal levels and thus shift the arterial pressure to higher levels.

GROLLMAN Yes I have also noted the absence of pathological changes at autopsy of dogs with so-called neurogenic hypertension of long duration This is evidence against the condition being hypertensive cardio vascular disease of the type encountered clinically I consider it as a reflection of a labile blood pressure rather than as hypertension

PERERA Do you ever see bilateral nephrectomy fail to produce hypertension? I am thinking of four patients with periarteritis nodosa who had proteinuria progressive renal damage oliguria then anuria uræmia and death and throughout their course had no hypertension at all

GROLLMAN I would explain your observation as indicating that the process did not involve the specific function of the kidney concerned in the maintenance of the normotensive state

JIMENEZ DIAZ We have seen in the clinic in some cases of nephrogenic hypertension that sympathectomy produces a violent decrease in blood pressure very difficult to control which lasts for many hours Other authors have reported similar findings

EXPERIMENTS ON THE RÔLE OF VASOCONSTRICTOR SUBSTANCES IN THE MECHANISM OF RENAL HYPERTENSION IN DOGS*

PAUL GOVAERTS

For many years, one has questioned the possibility that a lesion limited to the kidney might provoke and maintain a high blood pressure This question was settled by Goldblatt who, by constricting the renal arteries in the dog produced a permanent hypertension Although these memorable experiments were done twenty years ago, the mechanism by which the constriction of the renal arteries produces a lasting arterial pressure is far from being understood

Fifty five years ago Tigerstedt and von Bergmann found that the normal kidney, when crushed and extracted with saline, liberates a pressor and vasoconstrictor substance, renin Some of their experiments hinted at the conclusion that the kidney when poorly irrigated may liberate some renin Finally these investigators proved that the response to the pressor action of renin is greatly increased by nephrectomy These early observations were repeatedly confirmed but so far as the explanation of experimental renal hypertension is concerned they provided us with more pitfalls than clues Indeed when in the course of any experiment concerning the renal origin of hypertension the kidney is manipulated, artificially perfused or temporarily grafted to the neck of another dog the danger exists that these experimental procedures might squeeze renin out of the kidney In those conditions if the grafted kidney has been taken from a hypertensive animal, one may be led to the erroneous conclusion that the kidney "*in situ* and before any manipulation

*Aided by a grant of the Josiah Macy Jr Foundation New York

was excreting into the blood a pressor substance which was the cause of hypertension

This is why in 1945 (Govaerts 1945) I questioned whether experiments of this type (not excluding my own) demonstrated anything more than several ways to squeeze renin out of a kidney I decided to return with Dr Vermiory and Dr Lebrun (Govaerts *et al* 1950, 1951 1952 1953) to the fundamental question does the kidney liberate pressor substances into the blood during the course of experimental renal hypertension induced in the dog by constricting the renal arteries with Goldblatt clamps?

In order to avoid any manipulation or circulatory disturbance of the kidney which might cause a release of renin the blood was collected by the method of vascular catheterization which cardiologists use in man

As it was difficult to reach the renal vein by catheterizing the jugular vein we decided to collect blood from the vena cava above the mouths of the renal veins This is quite an easy procedure and can be done repeatedly on the same animal Thus the catheter was introduced under local anæsthesia into a superficial vein of the hind leg and pushed forward under X ray control until the tip was close to the diaphragm

Fifty ml of blood were aspirated to which 0 5 per cent citrate and 1 mg of heparin were added This blood was slowly reinjected into the saphenous artery of the same dog by using a motor driven pump which gave a constant pulsatile output (about 5 ml per minute) (Fig 1)

The pressure in this injecting system was recorded by a mercury manometer attached to a side tube As the output of the pump was kept constant the injection pressure was influenced primarily by the changes in resistance inside the arterial and capillary network into which the blood was being injected Moreover the resistance to injection was modified not only by variations in the local tone of the perfused network but also by changes in the level of the systemic arterial pressure since the perfused network kept its anastomoses

with normally irrigated regions Therefore, the systemic arterial pressure was continuously recorded by a manometer connected with the right femoral artery

When the regular bleeding and reinjection of the test dog with its own blood had been kept going for some time and both local and general pressures were stabilized it was easy, after having collected as usual 50 ml of blood from the carotid

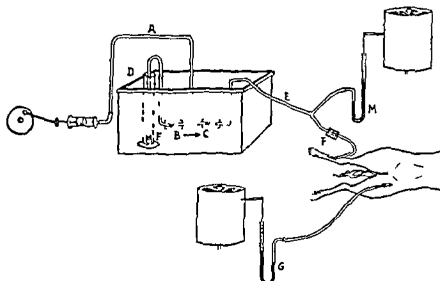


FIG 1 (See text) From Govaerts and Vermory (1949) *Bull Acad Méd Belg* 14 337

artery to substitute for that blood in the reinjection system, 50 ml of blood collected by catheterizing a hypertensive dog and to see whether there was any change in the injection pressure or in the general arterial pressure of the test dog The same procedure was repeated a little later with the blood collected by catheterization of a normal dog Finally the effects of renin and adrenaline were tested by adding to 50 ml of blood of the test dog ready to be reinjected into the saphenous artery a very small quantity of adrenaline (2 5 μg) or of renin (the saline extract of 50 mg of dry powder of dog's kidney) The test dogs, prepared as described

responded to this small quantity of adrenaline by a rise of about 20 mm Hg in the local perfusion pressure without change in the general arterial pressure. The response to renin was about 20 mm Hg and was nearly equal in both local and systemic pressures.

Preparation of Hypertensive Dogs

In order to get a condition of *acute malignant hypertension* the right kidney was removed and one week later the left renal artery was severely constricted with a Goldblatt clamp.

Ten dogs were prepared in such a way. The blood pressure at the time of the first catheterization three days after the second operation was increased by 15 to 30 mm Hg; the blood urea was very high (120–470 mg per cent). Four of these dogs died after four days (one with convulsions). The other six lived for ten to forty days and were catheterized more than once up to thirty five days. The blood pressure kept rising during their survival. Two of them developed lesion of malignant hypertension (hæmorrhagic suffusions in gastric and intestinal mucosa, retinal hæmorrhages).

Four dogs with both renal arteries constricted were used for studying the vena caval blood in the course of *chronic hypertension*. Their blood pressure was increased by 60 to 100 mm Hg and had been staying around 200–220 mm Hg for months. They were first catheterized after hypertension had existed for 35 to 100 days. The caval blood was collected once from two of them and respectively three and four times from the other two. Nine assays of vena caval blood were thus performed in the four dogs.

Results

The vena caval blood of 20 normal dogs produced no change either in the local resistance (mean +0.7 mm Hg $\pm$ 1.3)* or in the general arterial pressure (mean +2.9 mm Hg $\pm$1.6).

* $\pm$ = Standard deviation of mean.

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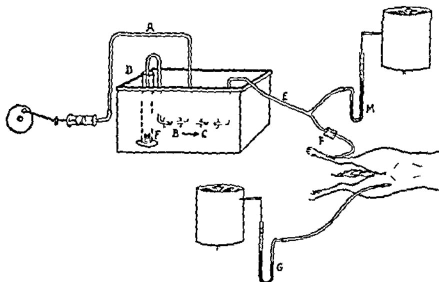


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On the other hand in our experimental results nothing indicates that chronic hypertension in the dog might be explained by the release of an increased amount of renin by the kidney Therefore the hypothesis was considered that an increase in reactivity to renin might explain chronic renal hypertension

The response to dog s renin was repeatedly tested over many months in dogs which were first normal and then made hypertensive by constriction of both renal arteries Renin was slowly injected into the femoral artery and the arterial pressure was recorded through the needle used for the injection Such a procedure needed but a femoral puncture and was performed without any pain in unanæsthetized dogs

After a preliminary period the dogs were made hypertensive by constriction of both renal arteries and the reactivity to renin was repeatedly tested During months of permanent hypertension brought about by bilateral constriction of the renal arteries the mean of the responses was frequently a little higher than the mean of the responses in the previous normotensive period but this difference was not statistically significant

These experiments do not provide any support for the view that an increase in responsiveness to renin might be the cause of chronic renal hypertension

When dogs either normal or having been hypertensive for 70 to 320 days were bilaterally nephrectomized the response to renin was enormously increased That change was statistically significant and it was greater in hypertensive than in normal dogs

The reason for the hyper reactivity to renin of nephrectomized dogs is still unexplained Our observations show that uræmia is not the cause of hyper reactivity Indeed when the main artery of the one remaining kidney was too severely clamped and the animal died after a few days with uræmia but still having a certain amount of surviving renal tissue the response to renin was unchanged during the period of survival Nevertheless the blood pressure was rising which

The vena caval blood of 10 dogs with acute hypertension (three days after constricting the renal artery of the one remaining kidney) had a local vasoconstrictor action (mean + 9 9 mm Hg ±2 2) It did not increase the systemic pressure in the test dog

The same local constrictor property was still present in the vena caval blood from four surviving dogs of that group when they had been hypertensive for ten to thirty one days

No change in the systemic or perfusion pressure was observed when using vena caval blood from dogs which had been hypertensive for periods from thirty five to one hundred days In nine experiments of this type the mean change in the perfusion pressure was +2 6 mm Hg ±1 9 against +0 3 mm Hg ±2 2 for the controls The difference is not statistically significant The same may be said of the change in general arterial pressure, the mean being respectively +0 6 mm Hg ±2 0 for the controls and +0 9 mm Hg ± 0 6 for the hypertensive group

It was necessary to make sure that the vasoconstrictor action of the vena caval blood in the early stage of hyper tension was not due to adrenaline This question was studied in a series of experiments which proved that the vasoconstric tor action of the vena caval blood was not suppressed by Benzodioxane derivatives (F 933), as was the constrictor action of a small dose of adrenaline added to control blood

Although our observations give no definite indication concerning the nature of the constrictor agent they are compatible with the view that a small amount of renin might be released by the kidney in the early stages of experimental renal hypertension They are in line with those of Fasciolo Houssay and Taquini (1938) Page (1940) Dell Oro and Braun Menendez (1942) and Haynes and Dexter (1947) who could detect the presence of a small amount of renin in the arterial blood of dogs in the early stages of renal hypertension According to Gollan Richardson and Goldblatt (1948) some renin might even be detected when the hypertension has lasted three months

Shorr (1948) has suggested that in the course of experimental renal hypertension the kidney liberates a substance (VEM) which potentiates the vasoconstrictor action of adrenaline

We have done some experiments on that point using the methods described above The same small dose of synthetic adrenaline (0 5 to 1 µg) was added either to 50 ml of carotid blood of the test dog or to 50 ml of vena caval blood from a hypertensive dog Both samples were tested by perfusing them into the saphenous artery network No difference in the constrictor effect of adrenaline was recorded when this substance acted either in normal blood or in the blood of a dog with acute or chronic hypertension

These observations do not support the opinion that potentiation of circulating adrenaline by a substance liberated by the kidney might be the mechanism of renal hypertension in the dog

At present we are repeating the same experiments with noradrenaline but so far our results are rather negative

Summary

When collected by catheterization without general anaesthesia or manipulation of the kidney the blood taken from the vena cava of chronically hypertensive dogs is devoid of any vasoconstrictor or pressor properties On the other hand the vena caval blood of dogs in the first stage of experimental renal hypertension has a weak vasoconstrictor property

This vasoconstrictor action is not due to adrenaline

Although there is some possibility of a small amount of renin being released by the kidney when the renal artery has been constricted there is no proof that such a small quantity of renin is active on the blood pressure except in a bilaterally nephrectomized test dog

The role of renin or renin derivatives in chronic hypertension lacks any objective proof and therefore must be considered as unlikely

Experimental renal hypertension in the dog cannot be explained by an increased responsiveness to renin

is a further proof that hypertension is not caused by an increased responsiveness to renin

In a group of six dogs which had been hypertensive for two to ten months bilateral nephrectomy did not lower the blood pressure during the three days which were the usual duration of survival

The results of our experiments in the dog thus lead to the same conclusions as the brilliant and careful experiments of Professor Pickering on the mechanism of chronic hypertension in the rabbit Our Chairman came to the conclusion that renin might be released by the kidney during a short period after renal artery constriction, but that in the chronic stage other factors come into play

On two other points our studies in the dog fully confirm those of Pickering (1945 and 1950) in the rabbit

(1) When the hypertension has lasted a long time bilateral nephrectomy does not bring the blood pressure down this is true for the dog as well as for the rabbit

(2) The increase in responsiveness to renin produced by nephrectomy is greatest when the kidneys are removed after the animal has been chronically hypertensive Therefore in the dog as in the rabbit it seems that the preparation most responsive to renin is a chronically hypertensive animal whose kidneys have been removed two days previously

Although in the dog during the early days after constricting the renal arteries a weak vasoconstrictor property may be present in the vena caval blood, I am not convinced that even at this stage a release of renin is the cause of hypertension The chief reason for my persistent doubts is that nearly all experiments aiming to prove that renin is released by the kidney have made use of nephrectomized test dogs No proof has been given that the small amounts of renin detected in such a way are sufficient to raise the pressure of dogs whose reponsiveness to renin has not been increased by nephrectomy

On account of these uncertainties over the role of renin we came to consider the validity of other hypotheses Recently

DISCUSSION

Braun Menendez No doubt the blood from the renal vein of the hypertensive dog has no action on the peripheral vessel nor on the blood pressure of the perfused animal But what is the minimal dose of renin that would have some action? Have you done any experiments with purified renin?

Govaerts No

Braun Menendez So you don t know if this lack of action is due to the absence of renin or to the presence of an amount of renin too small to show any action

Pickering I suppose the best control would be to infuse renin into the femoral vein in the animal in such quantities that it produced hypertension of approximately equal proportions and then to sample the inferior vena caval blood and see what sort of response you got on your test dog

Braun Menendez That is a quantitative experiment to see if the preparation is sensitive enough to renin The methods we use for detecting renin are different from yours We did the same experiment more or less by taking blood from the renal vein of kidneys transplanted into the back The blood was incubated with hypertensinogen and its extract was injected into the dog We found this method sensitive enough to detect renin in the renal venous blood in acute hypertension but in chronic hypertension we didn t find any That does *not* necessarily mean that no renin is present it may only mean that the method is not sensitive enough to detect it

Govaerts We found exactly the same thing

Heller There is I think another quantitative aspect which may be *purely formal* In a 10-kg dog 50 ml of vena caval blood represents say 3 per cent of the blood volume So if *in early hypertension* you get a weak pressor effect with this amount one might suspect that later the amounts of *pressor* substance have become subliminal That cannot be excluded though I *don t think it invalidates* the main argument

Govaerts You collect *blood* coming more or less directly out of the kidney and inject it into a *limited peripheral* network. So you can assume that if *there is an immediate* active constrictor substance issuing from the kidney and *potent enough* to give an action after it has gone through the whole system you could see the effect of such a substance when that blood is injected directly into a limited territory without dilution.

Heller The vena caval blood is already diluted to some degree but to what d gree? It would be more *convincing* if one took renal vein blood itself I cannot say how much the rest of the venous return *from* the body has diluted *the* blood

Page. I think it is extremely important to do the kind of experimentation which Prof Govaerts has done It illustrates the *paradoxical* situation we are in The *recent* experiments of Goldblatt and his group seem to show that angiotonin *at least* is present in the blood in chronic hypertension both in patients and dogs They feel quite strongly that

Bilateral nephrectomy increases the responsiveness to renin and this effect is maximal when nephrectomy is performed in a chronically hypertensive dog

In dogs which had been chronically hypertensive, bilateral nephrectomy did not lower the blood pressure during a survival of three days

There is no proof that in the course of experimental hypertension a substance released by the kidney potentiates the constrictor action of circulating adrenaline

Our observations do not support the hypothesis that during the stage of permanent hypertension the ischæmic kidney releases into the blood any substance with a direct and immediate constrictor action on the peripheral vessels

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weeks but after more than two months Nembutal lowered the blood pressure in these rats I am wondering if such experiments have been confirmed in the meantime because although it is only second hand proof it might throw some light on the mechanism of chronic hypertension

BRAUN MENENDEZ I have repeated them but not confirmed them

PAGE I too have repeated but not confirmed them

PICKERING Looking back Dr Braun Menendez on those early observations on the estimation of renin in the renal vein blood of your hypertensive dogs have you anything you would like to say?

BRAUN MENENDEZ We made estimations of renin in the renal venous blood and we found increased renin in acute hypertension and no increase in renin in chronic hypertension

PICKERING Didn t you take it from an explanted kidney?

BRAUN MENENDEZ From an explanted kidney without anaesthesia We found moreover that the amount of renin present in the renal venous blood was approximately the same as was present in arterial blood This was a surprise to us but that was what we found It is easy to explain this fact if one takes into consideration the magnitude of the renal blood flow and the relatively slow destruction of renin in the organism Based on this fact we abandoned our experiments on kidney explantations and estimated renin in the arterial blood of our chronic hypertensive dogs

they have demonstrated this The rest of us I think are a little uncertain still about the separation from the blood So on the one hand you have evidence both old and recent which strongly suggests the presence of angiotonin in the blood and on the other hand another type of evidence with which I think most people agree that with this quantity of blood and under these particular circumstances you find none All we can say at the moment and I agree with Dr Braun Menendez is that perhaps the methods are not sensitive enough The evidence simply isn t available to decide the place of angiotonin in the mechanism of hypertension Naturally with Dr Braun Menendez we feel a parental interest in the fate of angiotonin and renin hardly to be expected to be shared by others

BING Has anyone tried to do studies on the fluid from the artificial kidney, or perhaps the fluid obtained by intermittent peritoneal lavage? You could gather the fluid for a long time and study the pressor action

BRAUN MENENDEZ Skeggs and co workers have done it repeatedly in dogs from blood or from a dialysate of blood They recovered a greater amount of hypertensin from the blood of hypertensive dogs than from normal animals

PAGE The results as they are published are very clear cut

WILSON In chronic hypertension? For how long?

HELMER The longest period of hypertension was about three months More was found in earlier stages

PEART Does anybody believe that the methods of extraction are specific or that the methods of assay of hypertensive substance are specific as published?

BRAUN MENENDEZ Skeggs determines the identity of hypertensin by its susceptibility to trypsin hypertensinase boiling at *p*H 12 etc

PEART I should have thought that the only thing you could say so far was that they had extracted a pressor substance which in some respects behaved like hypertensin

I think there is a further point in Prof Govaerts experiments that he is not really assaying renin as such but a mixture of renin and angiotonin He is incubating renin with blood which he is then infusing The point which arises concerns the time between collection from the inferior vena cava of your chronically hypertensive dogs and re injection is it precisely the same as when you have incubated 100 mg of dog renin with the blood?

GOVAERTS The time interval is the same for the blood taken from the vena cava as it is when in the controls renin is added to a sample of blood There is no difference either between the time interval for the blood taken from the acute hypertensive dog or from the chronic hypertensive dog The only thing I can say is that you get a smaller effect with the blood of the acute hypertensive than with the blood of the chronic hypertensive

BLIN In 1946 there was a paper by Ogden and his colleagues (Ogden L Collings W D Taylor A N and Trip I 1946 *Tex Rep Biol Med*, 4 14) showing that in renal hypertensive rats no blood pressure depressor effect could be obtained with Nembutal during the first few

from shocked animals or that recovered from nephrectomized animals after kidney extract injection the height of the sustained pressor response to autolysed or alcohol fractionated kidney extracts is related to their renin content

Although it has not been possible to prepare from kidney extracts sustained pressor material free from renin it has been possible to prepare renin practically free from sustained pressor activity Fresh hog kidneys or cat kidneys were ground in a Waring blendor with three volumes of 95 per cent alcohol and allowed to stand at 25 C for twenty four hours The alcohol was removed by filtration with suction and the residue on the filter was air dried The dried material was then passed through a powder mill This powder was a source of renin free of sustained pressor material as judged by pressure curves in nephrectomized cats or by failure to recover active sustained pressor plasmas after injection of large quantities of an extract of the powder into nephrectomized cats

Whereas autolysis of kidney tissue prior to making extracts and autolysis of kidney extracts prepared from fresh kidneys produced a material that gives a sustained pressor response autolysis of the alcohol treated kidney tissue did not yield a substance having sustained pressor activity Possibly either an enzyme which could convert some precursor into sustained pressor substance or a precursor of this principle was denatured by the alcohol treatment

Recently Haas Lamfrom and Goldblatt (1953) have reported the preparation of a highly purified hog renin Since the first step in their procedure was autolysis of the kidney tissue it was thought that it would be of interest to determine whether their purified renin would give a sustained pressor response Dr Haas sent us a sample containing 470 Goldblatt dog units per mg of protein This material elicited a typical sustained pressor response in nephrectomized cats

The sustained pressor principle and renin are proteins derived from the kidneys and have similar physical properties When animals are injected over a period of time with relatively crude kidney extracts the serum of these animals

THE RELATION OF THE SUSTAINED PRESSOR PRINCIPLE TO RENIN

O M HELMER

Shipley, Helmer, and Kohlstaedt (1947) reported the presence in blood of a principle that elicits a sustained pressor response in nephrectomized animals The principle is released into the blood stream in conditions in which there is *a diminished blood flow and (or) diminished blood pressure* within the kidneys Although renin is also liberated under these circumstances, the elevation of pressure caused by the injection of sustained pressor plasma is much greater than would be expected from its low renin content The contour and the duration of the curve of the pressor response also differentiate this principle from renin However attempts to separate the sustained pressor material from renin in the plasma have so far ended in failure

Crude kidney extracts appeared to contain both renin and sustained pressor material When such extracts were *injected intravenously into cats that had been nephrectomized* two days before, it was possible to recover relatively large quantities of the sustained pressor principle from the plasma (Helmer and Shipley 1947) Only very small amounts of renin were found in these plasmas Twenty per cent of the renin in the original extracts could be recovered from the liver when the liver was removed one hour after the end of the infusion of the kidney extract When extracts were injected into normal cats, neither sustained pressor principle nor renin could be recovered in appreciable amounts from the plasma

Kidney extracts that give a pressure curve similar to that of the sustained pressor principle can be prepared in two ways One is by autolysis under toluene the other by alcohol fractionation (Helmer, 1950) In contrast to plasma obtained

renin prepared from autolysed kidneys gives a pressor response similar to that produced by sustained pressor plasma

The sustained pressor material may be related to renin Their chief difference is that based on their ability to produce angiotonin (hypertensin) the sustained pressor principle causes a greater elevation in arterial pressure The sustained pressor principle may be the form in which renin is released by the kidney into the blood stream

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DISCUSSION

PICKERING I hardly dared to mention after Prof Govaerts paper that VEM was cropping up Now we ve got an even more intangible substance which only comes out of the left renal vein! As I understand it this substance is only demonstrable in the nephrectomized animal when you inject it

HELMER I didn t mean to imply that the sustained pressor material was obtained only from the left renal vein In the slide shown that was the case The left renal vein is longer so it was possible to obtain blood samples from this vein without as much contamination with vena caval blood Therefore in the majority of instances greater concentrations of pressor material were obtained from the left renal vein than from the right one The substance is only demonstrable in the nephrectomized animal with the quantities *given 2 ml* of plasma One of the reasons we used a nephrectomized animal for a test preparation when looking for pressor agents of possible significance in the aetiology of hypertension was that Solandt cross-transfused equal amounts of blood between hypertensive dogs and normotensive dogs and found no increase in pressure in the normal *In a similar experiment* with hypertensive and nephrectomized dogs the blood pressure of the latter dog was elevated As Braun Menendez suggested the nephrectomized animal is so much

neutralizes both principles Serums that have a high "anti body" titre for renin have a similarly high titre for sustained pressor principle

To determine whether sustained pressor principle was elaborated by the kidney in experimental hypertension, renal vein blood was obtained from rats made hypertensive by unilateral nephrectomy and constriction of the artery of the remaining kidney (Helmer and Tilden) The plasma obtained from this blood was tested for activity in pithed rats which had been nephrectomized eighteen to twenty four hours previously

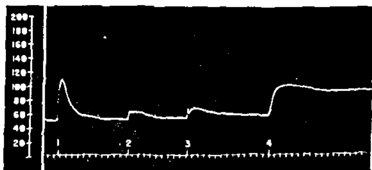


FIG 1 Mean blood pressure tracing of pithed cat nephrectomized two days before intravenous injection of (1) 0 2 unit angiotonin (2) 0 5 ml left renal vein plasma from normal cat (3) 0 5 ml of inactive recovered plasma (4) 0 5 ml left renal vein plasma from hypertensive cat Time marker—1 minute

An example of the pressor response to plasma derived from the renal blood of normal and hypertensive rats is shown in Fig 1

Sustained pressor activity was demonstrated in the blood obtained from the renal vein in eight hypertensive rats whose systolic pressures had been elevated for thirteen days to six months at the time the blood was drawn In the blood withdrawn from the left renal vein of seven normotensive rats the sustained pressor principle was not found Peripheral blood from hypertensive animals also was inactive

Summary and Conclusions

The sustained pressor principle and renin have physical and pharmacological properties that are alike Purified

inducing a shock state? It is possible to pith an animal without inducing shock characterized by the abnormally low blood pressures which were present in your animals

Helmer. Low pressures or pithing are not necessary for the substance to act An excellent response can be obtained in unpithed nephrecto mized dogs cats or rats with mean pressures of 100 mm.

Govaerts Does the blood of a dead cat produce a transfusion effect in a normal cat?

Helmer We have never given large amounts of blood from a dead cat into a normal cat Quantities of 2–4 ml do not cause a sustained rise in pressure in normal animals

Govaerts It seems to me that if such a substance has a rôle in hypertension it ought to be active when the blood is transfused

Helmer The answer to this question may be given by quoting Solandt s work which I mentioned earlier Large transfusions of hyper tensive blood into intact normal animals do not cause an elevation of pressure I believe blood from human hypertensive cases also does not raise the pressure of normotensive patients. Therefore I do not feel the fact that sustained pressor substance is inactive in an intact animal eliminates it as a factor which might play a rôle in the ætiology of hypertension

Govaerts My point is this is your substance present only in *extracts* of blood?

Helmer No You can inject whole heparinized blood or plasma

Govaerts But what if you inject the dead cat s blood into another cat?

Helmer If the cat is nephrectomized you get an elevation of pressure If it is not nephrectomized you don t

Floyer You say that if you tie a tape around the renal artery and then pull it tight the response to the sustained pressor principle increases markedly in a short time?

Helmer Within about half an hour or an hour

Floyer What happens if you put a Goldblatt clamp on the renal artery and only partially occlude it do you get the same effect?

Helmer We haven t done that Dr Braun Menendez you did an experiment years ago that has some bearing on Dr Floyer s question You have an illustration in your book showing results of an experiment in which you took blood from the renal vein of an acutely clamped kidney and injected it into a nephrectomized animal and got a typical sustained pressor response

Braun Menendez Exactly There is also the experiment of Houssay and Fasciolo in which they grafted an ischæmic kidney into the neck of a nephrectomized dog They observed a rise in blood pressure Secondly when the graft was removed the blood pressure remained high They never explained that but it can be explained in the light of your experiments

Floyer But does this substance which has a pressor effect in a nephrectomized animal but not in a normal animal have any effect in an animal with a Goldblatt clamp?

more sensitive I don t know what would happen in a normal animal if a continuous infusion of sustained pressor substance were administered

PICKERING You also mentioned that this substance was obtainable from the kidney after autolysis I wonder if you have any idea whether the amount in fresh kidney is more or less than the amount in autolysed kidney?

HELMER I don t know the relative amounts of the factor in fresh kidneys as compared to autolysed kidneys All I know is that autolysed kidney extracts give a typical sustained pressor response whereas extracts prepared from fresh kidneys do not give such a response

If fresh kidney extracts equivalent to about 13–34 g of original kidney are injected intravenously into a cat which has been bilaterally nephrectomized two days before blood drawn one to two hours after the completion of the injection has a high concentration of sustained pressor activity and a negligible quantity of renin Blood drawn from a normal cat treated the same way has no pressor activity in a nephrectomized animal So fresh kidney has a fair amount of sustained material or a precursor of it that can be changed to active material Again the presence of a normal kidney modifies the results

PEART Is there a dosage response curve for this substance?

HELMER Yes there is For instance if 2 ml of sustained plasma would give a maximum response smaller doses such as 0 5 ml would cause a much smaller rise in pressure Repeated smaller doses would produce a staircase like pressure curve until the maximum rise of pressure is attained that the individual cat can sustain

PEART Does it never come down?

HELMER Yes the pressure will come down as the physiological state of the animal deteriorates The duration of response varies from cat to cat The pressure may remain elevated in some cats for five to six hours in others only an hour Some cats will not respond at all in the same way that some cats will not respond to angiotonin

BARREDA Have you never found tachyphylaxis with your pressor material?

HELMER Not in a true sense One injection of sustained pressor plasma may cause an elevation of pressure lasting for hours However the sustained pressor substance will only raise the pressure to a fixed height in each animal Additional injections will not cause a further rise in pressure which will be maintained

BARREDA At the beginning of our studies we were not able to obtain the renin tachyphylaxis in the dog I wondered therefore if you never obtain it—or only do not obtain it with your substance?

HELMER Tachyphylaxis to renin in a cat depends upon the size of the dose injected Small doses repeated at a reasonable interval cause no tachyphylaxis One large dose will It is not due solely to the decrease in renin substrate Prof Pickering has maintained an elevated pressure in rabbits for days by continuous infusion of kidney extracts containing renin

GROLLMAN Can you elicit the same pressor effect in a normal animal or in a pithed animal in which the pithing has been done without

THE RÔLE OF THE KIDNEY IN THE MECHANISM OF EXPERIMENTAL HYPERTENSION

M A FLOYER

This paper may appear somewhat elementary and even outdated but I am sure that one of the main reasons for the comparative failure of research into hypertension lies in faulty understanding of the nature of the mechanism which operates in our experimental animals I wish to discuss the relation ship between the state of the kidneys and the blood pressure level in rats in order to see whether this will throw any more light on the mechanism of hypertension in these animals

I will begin by discussing the mechanism involved in the chronic stage of hypertension following partial occlusion of one renal artery and I will first discuss an experiment per formed by Wilson and Byrom (1939) which Dr Ledingham and I have confirmed many times Fig 1 shows serial blood pressure readings in one rat in this experiment After pre liminary baseline readings one renal artery is partially occluded by a silver clip I will henceforth refer to this operation as clipping the kidney and to the kidney as the clipped kidney The opposite kidney is not touched and will be referred to as the untouched kidney although this does not necessarily imply that it remains normal The blood pressure rises slowly and remains at levels well above normal If the clipped kidney is excised after a prolonged period of hypertension (in this instance twenty two weeks) the blood pressure remains raised after operation but if this is done after a short period of hypertension (three or four weeks) the blood pressure returns to and remains at normal levels Wilson and Byrom (1939) demonstrated that vascular lesions resembling malignant hypertension in man develop in the untouched kidney but do not occur in the clipped

Helmer We haven t done that

Flower Can you get this pressor principle from any other tissue besides the kidney or blood?

Helmer We have found renin in the liver of animals that have been injected with large amounts of kidney extract but have not tested these extracts for sustained pressor activity So the answer is no

weeks' hypertension the clip was removed from the renal artery resulting in a fall in blood pressure but not to normal levels (Fig 2) After a further period the series was divided in one group the kidney which had previously been clipped (now referred to as the unclipped kidney') was removed leaving the untouched kidney in which subsequent examina

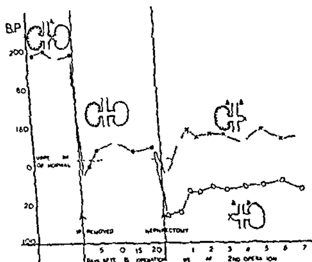


Fig 2 Mean blood pressure of a series of hypertensive rats following (1) *removal of the clip* and (") excision of (a) the *unclipped* and (b) the untouched kidney

tion revealed the presence of vascular lesions In these rats (Fig 2 upper curve) the blood pressure rose slightly after removal of the unclipped kidney and remained at hyper tensive levels In the remaining rats the damaged untouched kidney was excised leaving the unclipped kidney, which subsequent examination revealed as structurally normal in each instance in these rats the blood pressure came down to and remained at normal levels (Fig 2 lower curve)

I conclude from this that the blood pressure in chronic renal hypertension remains dependent on the state of the

kidney, they concluded that these lesions are the result of the hypertension and that the clipped kidney is protected by the presence of the clip They also concluded that these vascular lesions render parts of the untouched kidney ischæmic and that the ischæmic tissue is able to maintain hypertension in the same manner as the clipped kidney after removal of the latter On the other hand, it has also

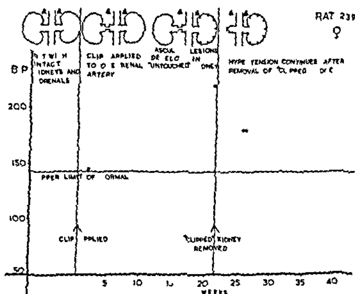


FIG 1 Blood pressure readings in one rat following clipping of one renal artery and subsequent removal of the clipped kidney

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been suggested that following a short period of hypertension, a change takes place elsewhere in the body which enables the hypertension to persist after removal of the clipped kidney

The first question which must be answered is whether *chronic* hypertension is always dependent on the state of the kidney or whether it is maintained by some other mechanism completely independent of the kidney

In the next experiment a series of rats were rendered hypertensive by clipping one renal artery After 20 to 40

confirms similar findings by Grollman Harrison and Williams (1943) in rats and by Pickering (1945) in rabbits

In a second series of rats with hypertension of similar height and duration to the first series the clip was removed from the renal artery (Fig 3 curve A) In each rat the blood pressure fell to normal levels in a few hours and remained

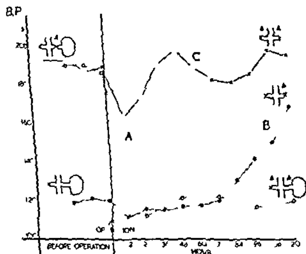


Fig 3

C Effect of total nephrectomy in hypertensive rats
A Effect of removal of the clip in hypertensive rats
B Effect of total nephrectomy in normal rats

normal for the rest of the animal s life This confirms similar findings by Byrom and Dodson (1949)

The contrast in blood pressure in these two series is very striking The fact that hypertension persists or even increases after total *nephrectomy must exclude* the possibility that a direct renal pressor substance is responsible for the chronic phase of experimental renal hypertension Dr Helmer has shown at this meeting that his sustained pressor principle will *maintain a raised* blood pressure *for many hours* in a nephrectomized animal but I find it difficult to believe

kidney even though hypertension has persisted for nearly a year This could be explained by assuming that the clipped kidney secretes a pressor substance and that this substance is also secreted by the ischæmic parts of the untouched kidney, excision of the damaged untouched kidney and restoration of normal circulation to the structurally intact clipped kidney prevents further secretion of this pressor agent and the blood pressure returns to normal On the other hand, we could also explain the results by assuming that hypertension is maintained by a pressor mechanism situated in some organ or organs other than the kidney and that this mechanism is inhibited by a normal kidney but can function in the presence of an abnormal kidney restoration of normal circulation to the structurally intact clipped kidney therefore results in the inhibition of this extra renal pressor mechanism and the blood pressure is restored to normal This is, of course the long debated problem as to whether renal hypertension in the chronic phase is maintained by a renal pressor substance or by some extra renal mechanism The following experiments were performed to study the question

A series of rats were rendered hypertensive by clipping one kidney and removing the other when hypertension had persisted for between two to twelve weeks the remaining kidney was removed After total nephrectomy the rats were maintained on a protein free and electrolyte free diet and were given 4–6 ml of 70 per cent glucose in water daily by stomach tube The mean blood pressure of the series is shown in Fig 8 (curve C) it can be seen that apart from a short post operative fall, the blood pressure remained at levels similar to those during the pre operative period The immediate post operative fall in the mean curve occurs because some rats appear very susceptible to post operative shock In many animals the blood pressure does not fall at all but rises steadily to levels considerably higher than before operation, I have seen the blood pressure maintain a steady rise for as long as eight days after total nephrectomy This

days later it was well above normal in all but one animal When the three curves in this chart are taken together one cannot escape the conclusion that a normal kidney has the function of maintaining normal blood pressure and that hypertension develops when the normal kidney is rendered abnormal or is removed

I conclude that an extra renal pressor mechanism is operating in the chronic phase (after two weeks) of experimental renal hypertension in the rat and that this mechanism continues to operate after total nephrectomy but is inhibited when *normal* circulation is restored to a structurally intact kidney *The same mechanism comes into operation and raises the blood pressure after total nephrectomy in previously normal animals*

I will now return to the problem of hypertension in a rat with one clipped and one normal kidney If the above reasoning is correct it might be argued that these rats should never become hypertensive since the untouched kidney is initially normal and should prevent the development of hypertension The fact that such animals do develop hypertension almost as rapidly as those with a *single* clipped kidney can be explained in two ways either the mechanism is different and a direct renal pressor substance is secreted in these animals or else the clipped kidney is able to interfere with the function of the apparently normal untouched kidney and render the latter incapable of preventing the operation of the extra renal pressor mechanism The operation of a direct renal pressor factor in rats with *two* kidneys can be excluded by the next experiment (Fig 4) in which both kidneys were removed simultaneously in hypertensive rats (duration two to seven weeks) with one clipped and one untouched kidney the blood pressure remained steady or rose still further in most animals

In the *next* experiment (Fig 5) I have contrasted simultaneous removal of both kidneys with removal of the clipped kidney alone in rats with hypertension of short (two to four weeks) duration There is a marked contrast between the

that a pressor substance, of which the kidney is the sole source, can persist in the blood stream and can cause persistent or increasing hypertension for as long as eight days after total nephrectomy It is much more likely that an extra renal pressor mechanism is in operation before nephrectomy and continues unopposed after operation When, on the other hand, normal circulation is restored to a structurally intact kidney, this extra renal pressor mechanism is inhibited and the blood pressure falls in a few hours I conclude, therefore, that an extra renal pressor mechanism operates in the chronic stage of renal hypertension and that a direct renal pressor substance plays little or no part in maintaining the blood pressure at this stage

Ledingham (1951) and I (Floyer, 1951) have already shown that this extra renal pressor mechanism is closely linked with the adrenals and with salt metabolism, but I will not discuss this question further at present

This concept of an extra renal pressor mechanism which is inhibited by an intact kidney with normal circulation is supported by the experiments in which it has been shown that hypertension develops following total nephrectomy Grollman and Rule (1943) showed that the nephrectomized member of a pair of parabiotic rats develops hypertension but that the intact member does not This has recently been confirmed by Ledingham (1951) Grollman Muirhead and Vanatta (1949) have demonstrated that severe hypertension develops in dogs maintained by peritoneal dialysis following total nephrectomy, and Braun Menendez and Covian (1948) have made similar observations in rats I have shown that hypertension following total nephrectomy is affected by adrenalectomy and by variations in salt intake in the same manner as hypertension induced by clipping the renal artery (Floyer, 1951) I have included in Fig 3 (curve B) the mean blood pressure of a series of normal rats maintained by peritoneal dialysis after total nephrectomy No rat in this series developed hypertension until three days after nephrectomy, but after this the blood pressure rose steeply and two

persistent hypertension following total nephrectomy and the sudden fall to normal when the clipped kidney alone is removed At post mortem the untouched kidneys were found to contain few if any, vascular lesions we again see that the blood pressure remains above normal after total nephrectomy but falls suddenly to normal levels in the presence of an intact kidney We must now ask why this intact untouched kidney does not prevent the initial rise in blood pressure the simplest answer is that the clipped kidney brings about some temporary change in the untouched kidney which renders it functionally abnormal and unable to prevent the operation of the extra renal pressor mechanism The most obvious explanation is that this is brought about by the secretion of a substance from the clipped kidney which causes vasoconstriction in the untouched kidney without necessarily having a direct pressor effect on the animal this vasospasm is released after removal of the clipped kidney There is slight evidence for the existence of such a substance, since Gordon and Flasher (1951) have demonstrated that venous blood from a rabbit kidney subjected to total ischæmia for half an hour will cause blanching in the opposite kidney without raising the blood pressure

When we compare the effect of total nephrectomy and of removal of the clipped kidney alone in rats with hypertension of longer duration (five to seven weeks) we again see that hypertension persists after total nephrectomy (Fig 6) After removal of the clipped kidney alone however the blood pressure frequently remains above normal though at a lower level than before If the same experiment is repeated in rats with hypertension of still longer duration the blood pressure after removal of the clipped kidney alone remains higher still It can be seen that a permanent change gradually occurs in the untouched kidney which renders it incapable of restoring the blood pressure to normal after removal of the clipped kidney It appears that the greater the duration and degree of pre operative hypertension the higher the blood pressure remains after removal of the clipped kidney Since as

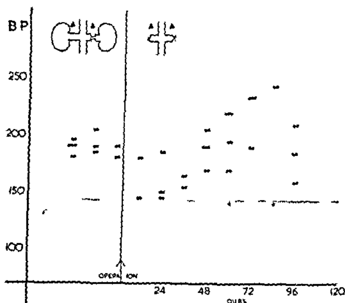


FIG 4 Effect of bilateral nephrectomy in hypertensive rats

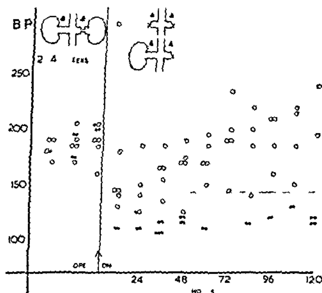


FIG 5 Comparison of removal of the clipped kidney alone (●) and removal of both kidneys (O) in rats with two to four weeks hypertension

of normal circulation to a normal kidney This suggests that, in a rat with one kidney partial occlusion of the renal artery has an effect no different from removal of that kidney in both instances the extra renal mechanism is allowed to operate and raises the blood pressure It is still possible however that a direct renal pressor substance is responsible for the initial rise of blood pressure and that the extra renal mechanism comes into operation a short time later This possibility is supported by the observations of Pickering (1945) that in rabbits total nephrectomy restores the blood pressure to normal in acute hypertension (one week) but not in the chronic phase (eight weeks) I have tried to repeat this experiment on rats the blood pressure nearly always remains raised after total nephrectomy in animals with hypertension of two or more weeks duration but in acute hyper tension the effects are more variable If the kidney is clipped and total nephrectomy is performed when hypertension has persisted for two or three days the blood pressure in some animals continues to rise but in others it is restored to normal sometimes total nephrectomy restores normal blood pressure when hypertension has been present for as long as ten days It is true that rats with acute hypertension appear more susceptible to shock but I would not like to explain these variable results by this alone It is possible however that further improvements in technique will bring more exact results

I have attempted to answer this problem in a different way I have tried to compare the rate of rise of blood pressure after clipping the kidney with the rate after total nephrec tomy Following total nephrectomy the blood pressure does not rise until the fourth or fifth day if the effect of clipping the kidney is the same as that of total nephrectomy the rate of rise after clipping would be expected to be similar to the rate after total nephrectomy The rate after clipping might be slower if the clip is applied too loosely to change com pletely the function of the kidney but it would only be more rapid if after clipping a different mechanism is operating

previously shown by Wilson and Byrom (1939), this change in the function of the untouched kidney occurs *pari passu* with the development of vascular damage, it is logical to suggest that following temporary vasoconstriction caused by and dependent on the presence of the clipped kidney, persistent ischaemia due to vascular damage gradually develops and permanently changes the function of the untouched

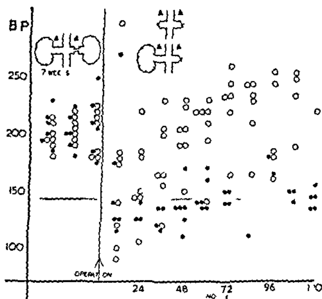


FIG 6 Comparison of removal of the clipped kidney alone (●) and removal of both kidneys (O) in rats with five to seven weeks hypertension

kidney Direct proof of this hypothesis can only be made by careful studies of renal function, but even at present it is difficult to interpret the experimental findings in a different manner

I will now discuss the mechanism which operates in the initial stages of hypertension (less than two weeks) I have *shown* that the mechanism in chronic hypertension (of more than two weeks' duration) depends upon the operation of an extra renal pressor mechanism and is inhibited by restoration

these rats are indicated by large circles and the mean blood pressure of the series by an interrupted line It can be seen that the mean blood pressure of this series is somewhat higher than that of the nephrectomized rats for the first three days after operation but that the curves follow a very similar path during the fourth and fifth days This suggests that a renal pressor substance may be secreted for the first three days after clipping the renal artery especially in those rats in which almost total infarction of the kidney has been produced On the other hand of the 16 rats with clipped kidneys which survived and which developed hypertension only in the six in which the blood pressure rose most rapidly was the rate of rise during the fourth and fifth days equal to but not greater than the rate of rise in the rats subjected to total nephrectomy This suggests that while a direct renal pressor substance may raise the blood pressure for the first three days after three day the blood pressure depends on the extra renal mechanism alone

I conclude that the phase during which hypertension in the rats is due to a renal pressor substance is of short duration possibly for only three days after clipping the renal artery and certainly for no longer than two weeks In rats in which the blood pressure rises slowly this renal phase may not occur but in animals in which there has been marked renal damage the renal agent is responsible for a rapid rise in blood pressure After this short period hypertension is maintained by the extra renal pressor mechanism similar to that which comes into operation following total nephrectomy

Summary and Conclusions

In the rat the raised blood pressure which occurs after partial occlusion of the renal artery is due to the action of a renal pressor substance only for a short period This period lasts possibly for three days only and is certainly no longer than two weeks In some animals in which the blood pressure rises more slowly this renal phase may not occur

Ten rats were subjected to total nephrectomy and were maintained by peritoneal dialysis, the blood pressure readings, taken twice daily, are shown by the dots in Fig 7 and the mean blood pressure of these rats is shown by the continuous line In a further 25 rats, in which one kidney had previously been removed, the remaining kidney was clipped and the rats

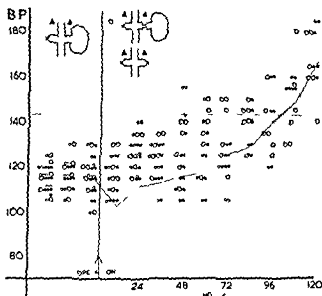


Fig 7 Comparison of rate of development of hypertension after total nephrectomy (●) and after clipping the renal artery (O = rats surviving more than five days after clipping o = rats dying within three days) ——— = mean blood pressure of 10 rats after total nephrectomy - - - = mean blood pressure of six rats which lived longer than five days after clipping the renal artery

were dialysed in the same manner as those subjected to total nephrectomy Ten of these rats did not develop hypertension within five days and are not shown on the diagram Nine rats developed hypertension rapidly but died within three days and at post mortem it was found that the kidney had been almost completely infarcted these rats are indicated by small circles The remaining six rats developed hypertension within five days and remained well for at least ten days

these rats are indicated by large circles and the mean blood pressure of the series by an interrupted line It can be seen that the mean blood pressure of this series is somewhat higher than that of the nephrectomized rats for the first three days after operation but that the curves follow a very similar path during the fourth and fifth days This suggests that a renal pressor substance may be secreted for the first three days after clipping the renal artery especially in those rats in which almost total infarction of the kidney has been produced On the other hand of the 16 rats with clipped kidneys which survived and which developed hypertension only in the six in which the blood pressure rose most rapidly was the rate of rise during the fourth and fifth days equal to but *not greater* than the rate of rise in the rats subjected to total nephrectomy This suggests that while a direct renal pressor substance may raise the blood pressure for the first three days after three days the blood pressure depends on the extra renal mechanism alone

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In the chronic stage (after two weeks), the raised blood pressure is maintained by an extra renal pressor mechanism (This mechanism is probably closely linked with the adrenal cortex and with salt metabolism or distribution) This extra renal mechanism comes into operation when the kidneys are removed or are rendered abnormal and is inhibited when normal circulation is restored to a structurally intact kidney no matter how long hypertension has persisted

Hypertension can occur in the rat when one renal artery is clipped although the other kidney is initially structurally intact The explanation is that the clipped kidney is able to affect the function of the untouched kidney (possibly by the secretion of a substance causing vasoconstriction in this kidney) *and to render the untouched kidney incapable of* inhibiting the extra renal pressor mechanism This change in the function of the untouched kidney is at first reversible, and removal of the clipped kidney after a short period of hypertension (up to about four weeks) restores the blood pressure to normal

When hypertension has persisted for more than about four weeks a permanent change occurs in the untouched kidney which renders it incapable of restoring the blood pressure to normal after removal of the clipped kidney the longer the duration and the greater the degree of hypertension the less the fall in blood pressure after removal of the clipped kidney This gradual change in the function of the untouched kidney occurs *pari passu* with and is possibly due to the development of vascular lesions caused by the hypertension

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DISCUSSION

PICKERING Dr Floyer you have given us a lot of food for thought Did you contrast the effects of removing the only ischæmic kidney in the first fortnight with the effects of its removal after a long time in the rat or the rabbit? I ask this because I confess to a slight prejudice in favour of thinking that something does come out of the kidney when you constrict the renal artery It seems to me one can t get over the fact that there is at least one pressor substance present in the kidney in the rabbit We found that if you had only one clipped kidney and you took it out at the end of a week the blood pressure came down to normal within four hours in about eight out of nine animals if I remember rightly I was very anxious to avoid what is loosely termed shock and I exteriorized the kidney so that it was a very slight operation just opening up the old incision under ether anæsthesia and putting a ligature round the kidney and hooking it out It didn t disturb the animal very much and the pressure did come down In rabbits after two months removing the kidney didn t bring the blood pressure down But the funny thing about the chronically hypertensive nephrectomized rabbit is that it gives the most peculiar response to renin as I think Prof Govaerts found in the dog In the chronically hypertensive nephrectomized rabbit the response to renin is very large and very long often lasting three to four hours

One of the things that is beginning to impress me in this meeting is the very peculiar behaviour of the nephrectomized animal which is apparently the only preparation that will respond to Helmer s sustained pressor substance and behaves in a remarkable way as Grollman has pointed out in developing gross hypertension I wonder whether these experiments on removing the kidney aren t in some way introducing two things first perhaps removing whatever the disturbance is in the kidney and secondly introducing an alteration in the behaviour of the animal which is confusing the picture

FLOYER You mean that one might be replacing one mechanism by another? Against this is the fact that in rats if one carries out nephrectomy without any shock the blood pressure remains up the whole time I don t know if you found the same in your rabbit?

PICKERING Yes

FLOYER You get no fall at all On the other hand if you remove the kidneys of a normal rat the blood pressure doesn t go up for at least three days and yet Dr Helmer showed that in the dog even after half an hour you get a remarkable change in sensitivity to the sustained pressor principle I would expect if one mechanism replaced another that the blood pressure would come down dramatically as it does after removing the clip would remain normal for two to three days and would

then go up again But it doesn t it stays up the whole time—it even goes on rising It does suggest that the mechanism which maintains hypertension in the absence of the kidney is in fact operating before nephrectomy

HEYMANS Did you try to denervate both kidneys before clamping the renal arteries or denervate the normal kidney while clamping the artery of the other one? Alterations in blood supply to one kidney may perhaps induce reflex vasomotor changes in the other kidney

FLOYER I have tried denervating both kidneys and it doesn t make any difference at all In fact the same thing was done by Fisher and Drury, they were able to show that this substance caused blanching of the opposite kidney even when it had been denervated

HEYMANS It must be a humoral mechanism then?

FLOYER Presumably yes

HELLER It would be very difficult to do in a rat but in a dog one could do clearance tests on the kidney and see whether you get any finer *indications as compared with histological changes*

FLOYER I am hoping to do that but I haven t yet been able to work out a satisfactory method for doing renal clearances on individual kidneys

PAGE What would you expect renal clearances to show you?

FLOYER I don t know

All these experiments were done on rats In the rat I think that there is this initial phase of purely renal hypertension for three to four days whereas in the rabbit it is presumably for a longer period I admit however that these experiments aren t really very conclusive Sometimes a more rapid rate of rise of blood pressure occurs after clipping the renal artery than after nephrectomy which does suggest that there is a transient phase of purely renal hypertension and that then after about three days the extra renal mechanism comes into operation

PEART What happens if you apply clips to both kidneys of a rat and then remove the clips? Wouldn't that help to decide partially about this question of vascular lesions? Both kidneys would be protected from the effects of the hypertension and you would expect that the *blood pressure would drop straight down* as soon as you took off the clips or even if you took off only one clip Does that actually happen?

FLOYER If you have one kidney clipped and the other removed the blood pressure comes down straight away after removing the clip I have never actually tried putting on two clips and removing both but I should imagine the same thing would happen

ETIENNE MARTIN Have you examined the vascular lesions in the livers of the rats with chronic hypertension?

FLOYER Yes you may get vascular lesions in the liver as in any other tissue but you never get much damage in this organ Frequently the liver shows no lesions at all

GROLLMAN I question the appropriateness of the term extra renal as you have been using it By analogy one might say that the diabetes which develops following pancreatectomy is extra pancreatic in

origin and that accordingly the pancreas was not responsible for the development of the diabetes which follows this operation

FLOYER I do not mean extra renal in the sense that it has nothing to do with the kidney but rather that the mechanism which elevates the blood pressure operates from an organ or organs outside the kidney If you remove the kidney the blood pressure goes up

GROLLMAN It may still be renal in origin in the same sense that the diabetes which follows pancreatectomy is pancreatic in origin

FLOYER The hypertension can t be due to a pressor substance secreted by the kidney It may be due to a pressor substance secreted from somewhere else or it may be due to absence of a substance which maintains normal pressure I don t know which One could say that rather than doing something actively to raise the blood pressure the kidney is failing to maintain normal blood pressure

BRAUN MENENDEZ Perhaps we could also explain these results by supposing that the extra renal change caused by clipping the renal artery is the same as by removal of the kidney That would explain the persistent hypertension after removal of the kidney

FLOYER Yes that s what I implied

THE EFFECT OF PARTIAL RENAL CORTICECTOMY ON THE BLOOD PRESSURE OF NORMAL AND HYPERTENSIVE ANIMALS*

JENS BING and PALI F SONDER

As it has been shown that the kidneys are involved in at least some of the different forms of experimental and clinical hypertension, it is of interest to know the effect of partial renal corticectomy on the blood pressure of normal and hypertensive animals That it is possible to keep dogs, rabbits and rats alive after partial corticectomy has been shown in some previous studies (Bing, 1949) In these the renal artery was clamped and the outer cortical layer was peeled off as uniformly as possible with a fine knife bleeding after removal of the clamp was checked by packing the kidney in moist gelatine sponge According to the amount removed it is possible to vary the degree of partial corticectomy The percentage remaining of the total renal tissue can then be assessed by means of a simple type of oncography (Bing, 1953)

After the operation the histological picture of the operated kidneys shows marked changes In the immediate post operative stages there is partial clogging of the tubules with casts, followed by connective tissue proliferation with atrophic and hypertrophic tubular changes In accordance with this picture, microdissection reveals atrophic aglomerular tubules and glomeruli with distended capsules which are thought to be the result of disruption of the corresponding tubules (Koefoed, 1949) This shows us that the partial corticectomy does not result simply in an extirpation of the outer zone of the cortex but gives rise to abnormalities in the rest of the kidney

*This work was carried out with support from King Christian X's Fund and Miss I A Brandt's Bequest

Since it has been shown that extensive partial or total nephrectomy may produce hypertension it is of interest to note that the blood pressure remained normal in six bilaterally and six unilaterally partially corticectomized rats and similarly in eight unilaterally corticectomized rats in which the other kidney was subsequently removed The blood pressure was also found to remain normal after partial corticectomy in two unilaterally nephrectomized rabbits

The effect of partial corticectomy on the type of hypertension which results in rabbits after removal of one kidney and clamping the other renal artery was studied by Sonder (1953) using the same methods for producing hypertension and measuring the blood pressure as in the well known studies of Pickering and his co workers (1951) It was first shown in three animals that a sham operation does not decrease the blood pressure of hypertensive animals Partial corticectomy, performed at the same time as the clamp was placed on the renal artery produced no rise in blood pressure in two cases (Fig 1) and this is believed to be due to the corticectomy However the clamp may have been too wide In eleven cases of what can be called the early stage of the hypertension the partial corticectomy being performed between the eighth and the thirty fourth day after the onset of hypertension the operation resulted in a sudden decrease of the blood pressure to normal values (Fig 2) The values remained normal throughout the subsequent observation period which in three animals extended for more than six months In contrast to this it was found that six out of seven animals which were partially corticectomized in what may be called the late stage of the disease from 51 to 125 days after the onset of hypertension did not react with any change in blood pressure (Fig 3)

The same difference between the effect of the operation on what has been called early and late hypertension seems to exist in the form of hypertension seen in about 10 per cent of unilaterally nephrectomized rabbits Sonder has studied five such cases In one which was operated in the late stage

the high blood pressure remained unaltered, while four operated upon in the early stage reacted with a decrease in blood pressure Unlike the effect of corticectomy in the early stage of hypertension produced by clamping the renal artery, where the blood pressure remained normal after its decrease, the blood pressure fall was only temporary in rabbits

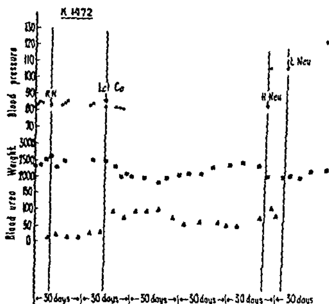


Fig 1 Clamping the renal artery of one kidney which at the same operation was partially corticectomized does not result in hypertension

R N right nephrectomy
L Cl clamp on left renal artery and partial corticectomy
R Neu and L Neu section of the buffer nerves
(From the thesis of Sønder)

with hypertension due to unilateral nephrectomy and in these a fluctuating course ending in permanent hypertension was found (Fig 4)

One rabbit, with hypertension caused by a clamp on the artery of the only kidney, was operated upon during the borderline period between the early *and the late stage of* hypertension, on the fortieth day after the onset of the

hypertension In this animal it was found that the blood pressure fell and remained normal for about eighty days after which time it again rose to the hypertensive level (Fig 5) If this is confirmed it shows that the second stage of the disease is not simply due to a continuation of the high blood pressure caused by the factor acting in the early stage

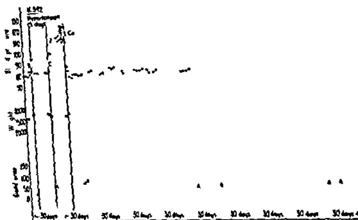


FIG 2 Partial corticectomy in a rabbit in the early stage of hypertension due to clamping the renal artery results in a sudden fall in the blood pressure to normal values

R N right nephrectomy
L C clamp on left renal artery
L.Co left partial corticectomy

(From the thesis of Sonder)

Another observation which needs confirmation in further studies is a finding in two rabbits each of which had one kidney partially corticectomized and the renal artery of the other kidney clamped in both cases it was found that clamping the renal artery resulted in hypertension of only short duration and that the blood pressure rose to hypertensive values when the partially corticectomized kidney was extirpated (Fig 6) If these results can be reproduced they show tha

the ability of the normal kidney to decrease the blood pressure is preserved also in the corticectomized kidney

After finding that the late stage of hypertension is uninfluenced by partial corticectomy, *Sonder studied the reaction* to a subcutaneous injection of a dose of the ganglion blocking hexamethonium bromide, "Vegolysin", using a dose, 20 mg

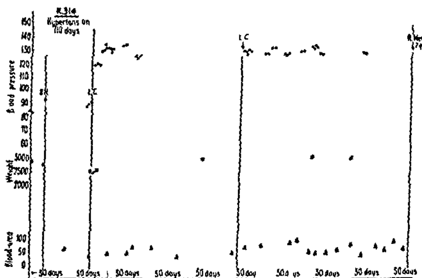


Fig 3 Partial corticectomy in a rabbit in the late stage of hypertension due to clamping the renal artery is without any effect on the blood pressure

R N right nephrectomy
L C clamp on left renal artery
L Co left partial corticectomy
R Neu section of the right buffer nerve

(From the thesis of Sonder)

per kg, which would not influence the blood pressure of normal animals The results of all his tests on normotensive rabbits and rabbits with hypertension after clamping the renal artery can be seen in Fig 7 It can be seen that the reaction is the same in the early hypertensive as in the normal rabbits, but increases in the later stage of hyper tension In rabbits with neurogenic hypertension caused by

section of the buffer nerves the Vegolysin test gave the same blood pressure fall as was found in animals with the more chronic hypertension and similarly partial corticectomy was found to be without effect on the neurogenic hypertension

Removal of the clamps from the renal arteries of animals with hypertension abolished the high blood pressure and in

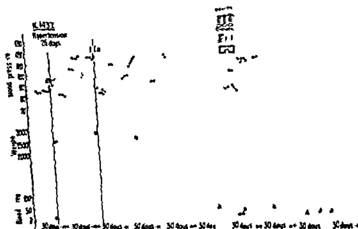


FIG 4 Partial corticectomy in a rabbit with hypertension due to unilateral nephrectomy results in only a temporary decrease in blood pressure which after about a week becomes strangely *fluctuating* ending in hypertensive values

R N right nephrectomy
l Co left partial corticectomy
Veg Vegolysin

(From the thesis of Sonder)

agreement with the studies of Blacket and Sellers (1951) it was found that the blood pressure decreases quickly in hyper tension of short duration and more slowly in hypertension of long duration (Fig 8) Although the blood pressure in the late stage of hypertension is *uninfluenced* by partial corticec tomy and reacts to Vegolysin which speaks in favour of a neurogenic mechanism these studies confirm that the kidney

the ability of the normal kidney to decrease the blood pressure is preserved also in the corticectomized kidney

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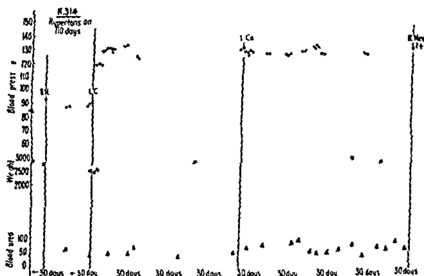


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the partial corticectomy normalizes blood pressure in the early but not in the late stage of that form of hypertension in rabbits which is due to clamping the renal artery

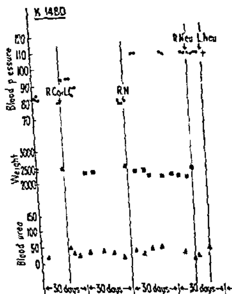


FIG 6 Partial corticectomy of the right kidney and clamping of the left renal artery is followed by a hypertension of short duration. Right nephrectomy results in increased blood pressure showing that the partially corticectomized kidney is able to counteract the hypertension.

R.Co + L.C right corticectomy and clamp on left renal artery
R N right nephrectomy
R.Neu section of the right buffer nerve
L.Neu section of the left buffer nerve

(From the thesis of Sonder)

This sign of a change of mechanism in the course of renal hypertension is in conformity with the earlier studies of Ogden Collings and Sapirstein (1946) Taquini (1950) Pickering (1951) and Covaerts and Verniory (1952) It is evident that

also plays a role in this stage of the disease, and show that in this respect there is no difference between a clamped but otherwise untouched kidney and a clamped partially corticectomized kidney

Conclusions

It can be concluded that partial corticectomy performed on normal rats and rabbits does not result in hypertension,

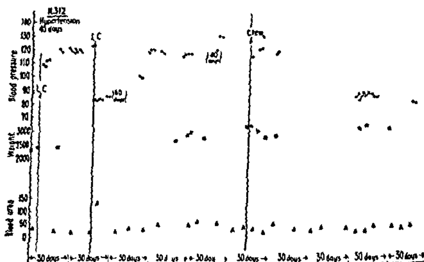


Fig. [illegible] Partial corticectomy performed at the borderline between the early and late stages of hypertension results in a decrease in blood pressure which remains normal for about eighty days after which time it rises to hypertensive values Removal of the clamp is followed by a decrease in blood pressure

L C clamp on left renal artery
L Co left partial corticectomy
C rem clamp removed

(From the thesis of Sonder)

showing that the factor which causes the hypertension after total nephrectomy found by Grollman (1951) Braun Menendez and von Euler (1947) and other authors is not caused by the absence of the outer part of the cortex

Secondly, it has been shown that clamping a partially corticectomized kidney will not result in hypertension, and that

counteract the rise in blood pressure caused by clamping the other renal artery

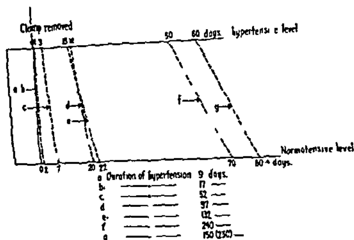


FIG 8 Removal of the clamp results in a decrease in blood pressure which occurs more quickly in the early than in the late stage of hypertension.

(From the thesis of Sonder)

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the early stage cannot be due to a deficiency such as that which occurs in totally nephrectomized animals, as partial corticectomy normalizes the blood pressure As the blood pressure remains normal in these rabbits, it would seem that the mechanism of the hypertension in the late stage is due to a different mechanism from that causing hypertension in totally nephrectomized animals

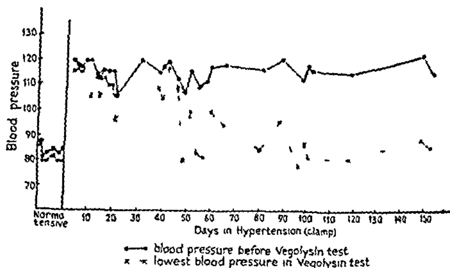


FIG 7 The blood pressure decrease after Vegolysin injection is equal in normal rabbits and in rabbits in the early stage of hypertension due to clamping the renal artery but much greater in the late stage of the disease

(From the thesis of Sonder)

In the form of hypertension occurring after unilateral nephrectomy corticectomy will decrease blood pressure for only a short period in the early stage of the disease It is without any effect on the blood pressure in the later stage of this form of renal hypertension or in the neurogenic hypertension due to section of the buffer nerves

Finally, it has been found in two cases that the partially corticectomized kidney like the normal kidney, is able to

blocking agent in the chronic phase do you regard them as indicative of a preponderance of the nervous regulation in the maintenance of hypertension?

BING Sonder found a different reaction in the acute and in the chronic phase This could be interpreted as some neurogenic factor acting but I wouldn t like to say more than that Moreover if the clamp is removed the blood pressure will go down again even in that stage of the disease

BEIN One can also argue that during the primary phase the action of the ganglion blocking agent is counteracted and afterwards there is just the normal action of the ganglionic blocker It is known that normally the higher the blood pressure the more the ganglion blocking agents lower it but if you elevate the blood pressure with a pressor amine the ganglion blocking agent will not act

BING I don t believe that could be the case because we have the same reaction in normal rabbits as in rabbits which are in the acute phase of hypertension. Would that not speak against it?

BEIN I m not sure

BING Then you must believe that there is a *factor in the blood* of normal animals acting against the ganglionic blocker

BEIN No because in the normal rabbit the blood pressure is not elevated

BING That might be the reason

DISCUSSION

PICKERING Have you controlled these observations by simply stripping off the capsules?

BING Yes

PICKERING What happens to the renin content of the kidneys that have had their cortex removed?

BING I don t know I tried to make such studies but I had no luck I attempted at first to remove exactly the same amount from the right and the left kidney of normal animals but I did not get this result and couldn t continue I had different amounts of renin from each of the two kidneys of the same rabbit Of course it should be possible to make such studies We have been interested in it especially because Taquini considers from his studies that the renin content is in the few outer millimetres of the kidney This seems very queer because morphologically there is no difference between that part and the other part only that there are fewer glomeruli We might ask Dr Braun Menendez about this problem

BRAUN MENENDEZ I have little confidence in the method of determination of renin that Fasciolo and Taquini used They incubated the renal substance with hypertensinogen and then tested the hypertensin formed in the Lowen Trendelenburg preparation of the toad a preparation which I don t like at all So I m really not sure about the quantitative aspects of this method Nevertheless they are careful workers and have made proper controls and they are quite definite about the point that most if not all of the renin found in normal kidneys is located in the outer layer of the renal cortex I tried to repeat these experiments in the rat but I wasn t satisfied with the method

PICKERING Then your renin ought to have gone completely Dr Bing That should be an easier thing to show quite apart from an accurate assay

BING Yes I must do that

FLOYER Dr Grollman and other people have found that total nephrectomy causes hypertension but there is always a lag of several days in the rat and rather longer in the dog and rabbit before the blood pressure goes up If you remove one kidney and corticectomize the other your dogs will presumably become uraemic like nephrectomized animals Have you kept them alive long enough to be able to observe whether or not hypertension develops?

BING I have done that in rats We had them quite uraemic and they had no high blood pressure

FLOYER Did you have any totally nephrectomized controls and did they develop hypertension?

BING I had no controls which were totally nephrectomized The animals were in such a poor state that I wouldn t place much trust in the blood pressure determinations

FLOYER It would be interesting to keep them alive with peritoneal dialysis and see if there is a blood pressure rise after partial corticectomy

BEIN Dr Bing with regard to your experiments with the ganglion

cells carried among them If then there is something of a cellular barrier round some or many ganglion cell this would mean that these particular cells might well prove relatively inaccessible to an injected quaternary salt (because of the impermeability of cells to such compounds) The practical consequence of course is simply that one cannot assume if one has blocked one ganglion that other ganglia will be blocked to the same degree

One must be careful that the blocking agent used is specific Any drug sufficiently like acetylcholine to compete with it at the ganglion synapse is liable to have actions related to acetylcholine at other places Thus one sees with *d tubo curarine* action at the neuromuscular synapse a feeble atropine and anti cholinesterase action as well as the ability to release histamine (which is possessed by a good many bases) *Nicotine* is somewhat better but possesses significant stimulant as well as blocking actions and is active at the neuromuscular junction

Tetramethylammonium is better still but has a number of curious actions a curare like effect the eliciting *of paraesthesiae* and the stimulation of noradrenaline secretion *Hexamethonium* is a good deal better if one defined a specific drug as one in which the principal action was exerted in a dose 100 times smaller than any other action it would pass the test This is one of the most important facts about hexamethonium and it seems to be more or less true of the other compounds related to it In one special respect almost all these compounds are specific in that they penetrate only very slowly into the central nervous system this means that one can largely discount central actions in interpreting their effects

It must also be remembered that ganglion block will paralyse the efferent side of the buffer nerve system It is this that accounts for the potentiation of the actions of adrenaline and noradrenaline or even of acetylcholine on the blood pressure and it is obviously liable to modify the response to many drugs

An important point is that the effect of hexamethonium

THE USE OF GANGLION BLOCKING AGENTS IN RELATION TO NEUROGENIC FACTORS IN HYPERTENSION

W D M PATON

One of the major dangers in the study of hypertension seems to be the ease of making misleading inductions, i e believing things to be causally associated which are not so in fact It recalls the story of the little boy living by the seaside on the border between Ulster and Eire who used to play with a small girl on the other side of the border They were only children and it was a lonely neighbourhood One hot day daringly they decided to bathe without bathing suits As the little girl undressed an expression of amazement crossed the little boy s face Gosh!" he said I didn t know that Catholics and Protestants were so different " Confronted with such complexities, I would like to avoid the wider aspects of hypertension and simply to examine in some detail a much narrower point how to obtain from the use of ganglion blocking drugs information about the neurogenic factors in hypertension

First, there are some special features of ganglionic block which have to be mentioned Ganglia differ in their sensitivity In cats for instance the ganglia supplying the nictitating membrane are more resistant than those supplying the salivary gland In man after a dose of hexamethonium one subject will faint on standing without there having been any effect on his eyes while another has his accommodation paralysed without any fall in blood pressure on standing There is no established explanation for this But if microscopical sections of ganglia are examined they obviously do not conform to the usual simple diagram they consist instead of a very complex basket work of dendrites surrounding the ganglion cell with many small supporting glial

genic component Obviously any tests must be done on a supine patient so that one studies the hypertensive process (rather than the postural reflexes) Since hexamethonium has only a slight or no effect in normal subjects lying down one could then attribute the fall seen in hypertensives to removal of *abnormal* autonomic tone

But clearly one would like to make this quantitative Three types of test suggest themselves —

(a) To determine *the threshold dose* in which one would assess the intensity of the autonomic drive by finding out how far it had *sensitized* the ganglia This is attractive because it is very safe and would not be complicated by reactions to a big fall in pressure but it would be liable to individual variations in sensitivity

(b) To determine *the maximal fall in blood pressure* obtainable by ganglionic block increasing the dose of blocking agent until no further effect was obtained This is attractive theoretically because with a big dose allowed to act for sufficient time it is unlikely that any significantly active ganglia would be spared Variations in sensitivity between subjects might thus be minimized but of course the method would have its dangers particularly in elderly people

(c) *The slope of the dose response line* Professor Pickering has suggested on the basis of the dose response curves obtained by Morrison and myself (1953) that the different slopes might correspond to different degrees of neurogenic activity In these particular experiments this may well not be the case since they were based on standing systolic blood pressures and probably measured the capacity of the venous bed the muscle tone and the vigour of postural reflexes as much as anything else But with a supine subject it is likely that the bigger the maximal fall obtainable the steeper would be the line relating to the fall in blood pressure produced to dose of hexamethonium given Combined with a measurement of threshold dose this might provide a most useful measure of autonomic tone in hypertension

One is uncertain how these simple tests compare with th

depends particularly on two factors the rate of excitation of the ganglion, and the duration of excitation Firstly, the block is much greater the faster the rate of stimulation Secondly, if you compare the initial peak postganglionic response with the response at the end of a minute's excitation, whereas with the normal ganglion the excitation rate must exceed 25 shocks a second before fatigue starts, in the partially blocked ganglion the fatigue begins much earlier, and fatigue is actually detectable, after a large dose of hexamethonium, even at a rate as slow as half a shock per second This means that if you determine the rate of excitation at which the maximal postganglionic response can be obtained, a rather startling "law of diminishing returns comes into operation, whereas the normal ganglion can yield an increasing postganglionic discharge with increases of stimulation rate up to 25 per second, the deeply blocked ganglion merely becomes weaker if the rate of discharge is accelerated beyond half a shock a second

These results have two implications first that the more active a ganglion is, the more sensitive it will become This may explain the considerable response one sometimes sees with very small doses of ganglion blocking agents in a hypertensive patient, second the only reactions to a substantial block which are likely to be able to show themselves are either non ganglionic ones, or else the activation of new ganglionic pathways at a slow rate which might produce only a transient effect *From this it seems probable that the tolerance developed to hexamethonium is largely humoral* If the superior cervical ganglion which is quite a resistant ganglion can be paralysed more or less completely to even so slow a rate as *half a shock a second it is unlikely that any ganglion* in the body will fail to be paralysed by the large doses of hexamethonium used in the tolerant patient

Tests for the Neurogenic Component in Hypertension

Having armed oneself with a specific ganglion blocking agent, the question arises of how to use it to assess the neuro

best method but it might well be that a brief infusion is the best way to reach a reproducible level of hexamethonium in the blood

PAGE We give an infusion until we get a fall in the supine pressure of at least 30–50 mm Hg Then we stop and use that dose to begin with We repeat the test in the semi reclining position and also determine how much it takes to produce postural hypotension

PATON If in fact the hypertensive process is divided up as some of the evidence suggests into a neurogenic element (whether it stems from the kidney originally or not) and a humoral element (wherever that stems from) the prognosis of the patient may depend on that initial division A worthwhile approach therefore would be to try and assess such patients in these terms and see what happens later There may be in fact an unrecognized variety of types of hypertension

PAGE There is certainly a variety of response to hexamethonium. And there is a small number of patients in whom the neurogenic element is a very potent one Not more than 20 per cent show that greatly heightened effect which mimics those seen in dogs with experimental neurogenic hypertension

PATON How does the fate of the 20 per cent compare with that of the remaining 80 per cent

PAGE So far as anybody can see it doesn t seem to be very different Of course I m talking about severe hypertension—in other words severe enough to treat the patient with so dangerous a drug

McMICHAEL. There are difficulties aren t there in using supine blood pressure? As Paton has shown the response to methonium varies with the intensity of stimulation of the ganglion Often you get very little effect at all on the blood pressure in the supine position but a tremendous drop in pressure when the patient stands up It looks as though ordinary tonic impulses may not be blocked but when reflex volleys try to pass through the ganglia which probably occurs when the patient stands up these are blocked and the pressure falls parallel with the postural drop in cardiac output That may be too crude an explanation

ROSENHEIM Isn t the term neurogenic tone rather too simple? If you imagine a series of impulses coming down a vasoconstrictor nerve they are going to hit an arteriole which may be in varying states of humoral tone The same neurogenic stimulus may have a far greater effect on an arteriole already constricted by humoral agents than on one in which there is little humoral effect

PATON Yes I m aware that it s crude certainly But I get the impression from what has been said so far that there is no decisive evidence for neurogenic tone in human hypertension at all Yet in fact everybody talks about it What I am trying to suggest is that it is better to use even a crude measure than no measure at all For instance if most hypertensives supine don t get a fall with hexamethonium then it implies that the neurogenic element is rare In that case success with hexamethonium clinically must depend on the fact that the postural reflex is a vigorous one in the hypertensive as well as in the normal

ROSENHEIM I would have said that most hypertensive patients when supine did get a fall with hexamethonium

tests using tetraethylammonium or serotonin that Dr Page has mentioned But I am confident that the factors mentioned above are important and that a test of the kind outlined could be useful In any case it seems a wicked waste now that we have a fine population of reversibly sympathectomized patients, not to use them to try to put some precision into the much abused term 'neurogenic'

REFERENCE

MORRISON, B and PATON, W D M (1953) *Brit med J* 1 1299

DISCUSSION

PICKERING Dr Paton the difference between your experiments on the nictitating membrane and the experiments which you propose in man is that in the former you have a fairly simple preparation If you record the arterial pressure in man as an index of the state of vasomotor tone you have the complications of things like cardiac output haven t you?

PATON I would say that that is an unanswerable criticism At the same time I have been struck that although ganglion block is rather a crude tool yet it still is a tool for assessing neurogenic elements Sedation was the same and simple bed rest I think ganglion block has advantages in that it is quick and you don t get all the changes due to rest in bed and so forth and you avoid some of the danger of using a non specific drug like amytal It would be interesting to try such tests systematically for a while There might be a better test for circulatory tone than simple blood pressure measurement perhaps hand blood flow or temperature in a skilfully chosen region of the skin

PAGE I don t understand the suggestion Are you suggesting using your test as a measure of neurogenic tone? It has been widely used hasn t it?

PATON I m not aware that it has systematically A lot of people have measured the fall of blood pressure in response to a test dose of hexamethonium say 50 mg but that is usually neither a maximal effect nor a threshold effect They often do it with patients sitting up or standing or they let him stand up every ten minutes or half an hour and so on But a systematic attempt to keep the patient supine and to find (for instance) the dose that will just produce a hypotensive effect would be a procedure which eliminates a certain number of variables and might give you a genuine quantitative measure of neurogenic tone

PAGE Do you mean give it by infusion?

PATON I am not sure what method would be best One would have to study the blood levels produced by different procedures to discover the

change and nothing you could do would ever alter it held back therapy tremendously Even in the days of sympathectomy at first everybody said one shouldn t touch the ones that have a fixed hypertension and it didn t work out that way at all Some of the most brilliant results were obtained in malignant hypertension

PLATT In this interesting spectrum of effects which Dr Paton showed us where does pentamethonium stand? Hexamethonium seemed to be almost ideal as a ganglion blocking agent with comparatively little neuromuscular effect but there was some and I wondered if pentamethonium had less and why one didn t use it What are the advantages of hexamethonium?

PATON They are very similar pharmacologically and they would certainly be indistinguishable in clinical use Hexamethonium is used most commonly because it is slightly more potent They originally seemed to have different relative actions on the parasympathetic and sympathetic but that difference has evaporated in clinical practice

PAGE If you produce experimental neurogenic hypertension pentamethonium or hexamethonium causes the pressure to come down quickly and then regain the control level relatively rapidly with the next dose it falls and rises and so on until you get what we call the chronic response pattern Whereas the renal experimental hypertensive behaves like a normal dog the first dose gives a fall the next dose a lesser fall and the third dose a rise in blood pressure the whole pattern is changed What do you think has happened to the ganglia in the experimental neurogenic hypertensive such that each dose of the blocking agent on the ganglia so quickly disappears?

PATON I don t believe that anything has happened to the ganglia If you give hexamethonium daily to a rabbit, for instance his ears continue to flush after the injection for a month at least

PAGE But this is a matter of minutes not days

PATON You can give the drug repeatedly to an anæsthetized animal without losing the ganglion blocking action.

PAGE Yes but in the normal animal or in the renal hypertensive animal you abolish within a few minutes its ability to have a blood pressure fall when you inject the blocking agent Whereas in the neurogenic hypertensive and in those patients that I showed blockade must be very transient which would suggest that there is a complete change of pattern And that would be the kind of patient that I would say was a neurogenic hypertensive Have you any explanation for this?

PATON No I haven t

PAGE I'm sure they do

PATON Yes but some don't I should have thought that it was most important to know whether neurogenic factors are really significant or not

WILSON Do these observations throw any light on fixation of blood pressure in a patient with essential hypertension which may occur before there is any evidence of papilloedema or renal involvement?

GROLLMAN One might look upon the phenomenon as a reflection of the intensity of the process as this becomes more severe, the rise in blood pressure becomes less labile and more permanent An analogy may be drawn from the diabetic state in which during the early stages of the disease there is neither glycosuria nor hyperglycaemia for these may occur intermittently only when the disease becomes more severe is this lability lost and these characteristics of the disease become permanent

WILSON Even so there must be an explanation of such a change

PAGE I am more impressed with the fact that if you average the blood pressure you don't get the lability The lability seems to me to arise chiefly because of the capricious measurements of blood pressure We take blood pressures twice a day and then average them every day, and over a period of weeks and months the averages show that the lability is really not impressive a trend becomes established which exhibits relatively few fluctuations I think we are impressed in the early stages of hypertension with the fact that one time single measurements are normal and the next time they are up We have been very insistent that one of the great difficulties in testing all new hypotensive agents is the fact that you must have average blood pressure measurements sufficient in number to establish a trend rather than single determinations even though they appear to be enough Even blood pressures taken year after year in office practice don't reflect changes that will occur when you want to conduct a reproducible experiment The important thing is to establish a trend If you average what seem to be highly variable measurements you find the blood pressure goes up and swings down very slowly over periods of weeks and months Some people say you can do a control in two or three weeks but some patients you can't control in six months even following the averages

WILSON Yes but in so many patients this swing which I agree does occur just disappears and then sometimes over the course of a few months the blood pressure becomes remarkably fixed at a high level This is particularly true when renal hypertension enters the malignant phase but may occur in the absence of the latter

PAGE You do see the swing even though it may be more occasional even in the very severe hypertensives especially when you bring them into hospital

I agree with you in that I would rather treat a patient with a labile high pressure than one that is pretty fixed But on the other hand I think it is equally true that even though they are fixed the therapeutic response is very often extremely good The dictum that held for so many years that once the blood pressure was fixed there was a structural

So far as survival is concerned there is as yet little difference between the groups possibly because the study has not yet lasted long enough ($\chi^2=3.5$ $p=0.6$)

Encouraging features are in the state of health of the treated group Ten out of eighteen were able to return to work though two of these died later In fourteen out of eighteen retinitis regressed Heart size diminished significantly in five cases and the electrocardiogram sometimes showed regres-

Table 1

MALIGNANT HYPERTENSION

Survivals July 1953

Time of survival (months)	Untreated	C 6 treated	(Still surviving)
0–1	10	–	(0)
1–3	9	6	( )
3–6	8	4	(3)
6–12	4	2	(0)
12–24	3	2	( )
Over 24	1	2	( )
	35	18	(9)

Untreated cases	Survivors 8 back in active life
All went downhill	Papilloedema cleared in all
Retinitis subsided in 1	*Fatal Cases* 5/9 retinitis improved [illegible]9 had temporary return to activity

sion towards normal Those in whom kidney damage had not elevated the blood urea beyond 100 mg showed no further deterioration of kidney function due to the drug and were in fact easier to manage than the others owing to delayed excretion and prolongation of the effects of single doses

Three of those patients have shown the development of what appears to be a chronic organizing fibrinous oedema of the lungs *This resembles in its* radiological distribution the condition frequently *seen in the* terminal stages *of* hypertensive left ventricular failure with uraemia (uraemic lung) Possibly prolongation *of* life leads to an unusual degree of

LESSONS FROM HEXAMETHONIUM STUDIES IN MALIGNANT HYPERTENSION

J McMICHAEL

Malignant Hypertension

Classification

Primary Rapid No antecedent hypertension
May begin in pregnancy

Secondary Complicating pre existing hypertension
Course may be slower

Nephritic Complicates pre existing nephritis or pyelo nephritis

Criteria of Diagnosis Grade IV fundus changes
B P very high usually
Signs of accelerating damage
(1) Renal (2) Visual (3) Cardiac
(4) Cerebro vascular

Treatment

Hexamethonium—deep subcutaneous or intramuscular injections to get *upright* blood pressure to a level short of giddiness and syncope Effect gradually wears off in six to eight hours, when injection is repeated (thrice daily) A recent alternative is a double dose at night with morning "hang over" and one further afternoon dose

The results are shown in the accompanying Table set against 35 untreated controls diagnosed by the same criteria in the writer's beds in the pre hexamethonium years Survival rates in this control group closely resemble those in Keith, Wagener and Barker's series (1939)

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Table I

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6–1	4	2	(0)
1–24	3	2	( )
Over 24	1	2	(?)
	35	18	(9)
Untreated cases All went downhill Retinitis *subsided in* 1		*Survivors* 8 back in active life Papilloedema cleared in all *Fatal Cases* 5/9 retinitis im*proved* 2/9 had temporary return to activity	

sion towards normal Those in whom kidney damage had not elevated the blood urea beyond 100 mg showed no further deterioration of kidney function due to the drug and were in fact easier to manage than the others owing to delayed excretion and prolongation of the effects of single doses

Three of those patients have shown the development of what appears to be a chronic organizing fibrinous œdema of the lungs This resembles in its radiological distribution the condition frequently seen in the terminal stages of hypertensive left ventricular failure with uræmia (uræmic lung) Possibly prolongation of life leads to an unusual degree of

organization of the alveolar exudate One patient with this lesion has recovered and is going on with treatment although the fibroid changes in his lung have added a further factor to his dyspnœa

In patients dying early in the disease gross fibrinoid necrosis was found in the renal arterioles and glomeruli This material takes a homogeneous red colour in Mallory preparations In a patient dying after eight months' control of arterial pressure, however, blue staining collagen was seen in the situations where fibrinoid material might be expected This may represent a healing stage of the disease but no firm conclusion on this point can be reached as yet

In the course of repeated blood pressure recordings made during this study some further points have struck us

(1) The blood pressure level and fluctuations in malignant hypertension often do not differ significantly from the levels seen in other examples of severe hypertension where retinitis is less severe or absent

(2) We have watched the development of malignant hypertension closely in two patients where the condition began in pregnancy Both were rapidly fatal four weeks and six weeks from the onset In one papillœdema developed a few days before death and in the other it was present early when the blood pressure was only 170/110 Both cases showed unusually extensive renal arterioloneerosis at *post mortem* It is difficult to say in these cases that the height of the blood pressure was the factor determining arteriolar damage though the tempo of progression may have contributed The whole picture was one of a severe vasculitis The course of this illness was entirely uninfluenced by blood pressure control

(3) Subsidence of the papillœdema and retinopathy does not exactly parallel blood pressure control In one patient at least the retinopathy and especially œdema of the disc spread for several weeks after the pressure was well controlled

About ten years ago I had a chance to watch the fundi of a patient who had an episode of acute hypertension in the

course of nephrocalcinosis (induced by self medication with alkalis for a duodenal ulcer) Papillœdema and hæmorrhages *developed* as the blood pressure was *subsiding*

I hope to provoke discussion by saying that the role of mechanical factors in the production of both arteriolonecrosis and papillœdema have been overstressed in this disease Some other agency is damaging the vessels High pressure may contribute to the damage but is not its sole cause These clinical conclusions find support in the work of Dr H A Fleming who is here today He found that in experimental renal hypertension in rabbits arteriolonecrosis was present in those which died of the disease but absent in those surviving though levels of pressure were comparable in the two groups

REFERENCE

KEITH N M WAGENER H P and BARKER N W (1939) *Amer J med Sci* 197 332

DISCUSSION

PICKERING I should like to say that *in a general* way my own experience fully bears out what Prof McMichael has said I won t offer any comments on the mechanism of the changes specific to the malignant phase but I would *like to say that* this peculiar change which he has described in the lungs we have also seen in one patient a chronic nephritic who had nitrogen retention before we gave him hexamethonium. He presented the clinical picture which Prof McMichael has described but which he didn t emphasize today which is quite unlike anything I ve ever seen before He was extremely breathless but his breathlessness was made much worse when he sat up I think you or Dr Morrison mentioned that in a *paper*?

MCMICHAEL Yes the patients notice that this pulmonary dyspnœa differs from cardiac breathlessness it is relieved by lying down in contrast with the breathlessness of left ventricular failure which is made worse by lying down

GOVAERTS I did not know of the possibility of that exudate which probably is aseptic occurring *in the lungs* but I am surprised that at the same time there is no similar exudate in the pericardium

MCMICHAEL It is independent of uræmic pericarditis

GOVAERTS The *fact that this* exudate seems to be strictly localized to the centre of the lung suggests that there might be a lymphatic factor

McMICHAEL Dr Doniach is very interested in this problem and he is quite puzzled by this distribution (Doniach, Morrison and Steiner 1954, *Brit Heart J* in press) You may sometimes see an actual line of demarcation parallel to the surface There must be some anatomical reason for this perhaps there are no bronchial pulmonary artery anastomoses beyond these limits

GOVAERTS It is very curious that there is no connection with an exudate in the pericardium

McMICHAEL. Although it was once called uræmic lung it is in fact a type of manifestation of left heart failure because it can occur in many patients without uræmia at all

PICKERING The clinical picture of these patients is quite different from left ventricular failure because the one thing a patient with left ventricular failure wants to do is to sit up and the one thing these patients want to do is to lie down

McMICHAEL Yes but the attack may produce the œdema, and then later on organization takes place and you get a pulmonary type of dyspnœa from the fibroid changes

ROSENHEIM I should have thought that 'hexamethonium lungs though we haven t seen them in our series were quite different from the so called uræmic lung We have seen a patient with malignant hypertension and uræmia come in with a typical uræmic lung which cleared magnificently under hexamethonium This suggests a quite different pathology

McMICHAEL. Absorption depends on whether the exudate is albuminous or fibrinous A fibrinous exudate probably will not clear up

ROSENHEIM In your patients it developed while they were on hexamethonium and while they were responding to hexamethonium?

McMICHAEL. Yes

PICKERING Pneumonic fibrinous exudate of course would clear up?

McMICHAEL Yes

HEYMANS Hexamethonium is as shown by Dr Paton a fairly specific ganglionic blocking drug This blocking effect is however transient Administration of hexamethonium has thus to be repeated in order to maintain a blocking effect I should like to ask Prof McMichael why surgical excision of the sympathetic ganglia would not induce the same reduction of blood pressure as transient and repeated blocking of the ganglia with hexamethonium?

McMICHAEL Hexamethonium block must affect all ganglia whilst surgical excision never removes all ganglia In spite of repeated attempts at treatment of this condition by sympathectomy, the results are very disappointing I think about 10 per cent of good results is about as much as anybody ever got You can by pharmacological means by repeated doses of hexamethonium get a better reduction of blood pressure over longer periods of time than you get by surgical sympathectomy

HEYMANS Partial sympathectomy doesn t of course eliminate all the ganglia, whereas hexamethonium may block all the ganglia But complete sympathectomy as performed by Dr Grimson also removes

Fig 1a (Platt) Left eye × [illegible]

Fig 1b (Platt) Right eye × [illegible]

[To face page [illegible]



Fig. [illegible] (Hitt) Left eye [illegible]

[illegible] good results. I would [illegible] per cent [illegible] that have now been operated [illegible] did Dr. Kn[illegible] G[illegible] did. I [illegible] intelligent [illegible]. The question then arose in the [illegible] the effort [illegible] some of his cases, [illegible] an inflamm- [illegible] same difficulty [illegible] his good results [illegible] and I accept [illegible] per cent [illegible] the rest of [illegible]. But I think [illegible] a suffer- [illegible] in doing it [illegible] true, consequently [illegible] patients. But if you really [illegible] these so-called [illegible] I think we've got to find [illegible] that the genetic [illegible] find that, we're [illegible] results without [illegible]

[illegible] that Dr. Pace and [illegible] would be [illegible] depress the [illegible] in the same [illegible] I don't [illegible] system. [illegible] the cases which [illegible] depends on the [illegible]

[illegible]

Fig. 2b (Hitt) Right eye [illegible] 9-53

all ganglia. In totally sympathectomized dogs exclusion of the sino-aortic nerves no longer induces hypertension If hexamethonium produces a better reduction of blood pressure than surgical sympathectomy some other action of hexamethonium besides the ganglionic blocking effect may be involved

PATON But a surgeon can t remove all the ganglia because many of them are in the spinal nerves and quite inaccessible To remove these accessory ganglia which Skoog and Wrete and Boyd and Monro described as well as the obvious lumbar chains you would have to paralyse the man by sectioning most of his spinal nerves When patients are studied after fairly thorough sympathectomies you can find areas of skin which still sweat when exposed to heat particularly in the lower part of the body

MCMICHAEL. Henry Barcroft studied a series of sympathectomies of the upper limb done mainly for Raynaud s disease and found that the vessels had returned to full physiological reactivity nine months after the operation It s terribly difficult to achieve permanent and complete surgical sympathetic denervation

HEYMANS I wish to point out that at least in dogs complete surgical removal of all sympathetic ganglia from the stellate ganglia down to the pelvic ganglia doesn t leave any sympathetic vasomotor connection between the central nervous system and the periphery Experimental control did not show the presence of accessory ganglia or connections involved in the reflex regulation of blood pressure Regeneration and re-connection may however occur after sympathectomy

PLATT Could I show some slides? They are not as good as I would like but they do bring a more cheerful note into this discussion of hypertension Fig 1 shows the fundus of a boy of twelve with gross exudate in the macula who came to us with a diastolic pressure of 180 and Fig 2 is his condition today The boy s sight is now absolutely normal Hexamethonium was the only treatment he had and most of the exudate had cleared in a few weeks

JIMÉNEZ DIAZ Prof McMichael do you sometimes use hexamethonium in patients that have been totally sympathectomized if the high blood pressure persists?

MCMICHAEL. Yes we can with the same result as in other cases. There is some suggestion that sympathectomized patients are rather more sensitive perhaps because more impulses are going through the remaining ganglia

HEYMANS Totally sympathectomized dogs recover a normal arterial pressure and are able after a certain length of time to regulate their blood pressure We have suggested that more peripheral sympathetic ganglia may compensate for the removal and disconnection of the ganglia [illegible] cated in the sympathetic chain

[illegible]AGE I agree with Dr Heymans on this problem Some years ago [illegible]hen we were doing sympathectomies we were taking all comers There was no method of selection and we were obviously operating on an inhomogeneous population of hypertensive patients When we were doing anterior nerve root sections the fact emerged that some people

got remarkably good results and some remarkably poor results I would go a little higher than 10 per cent I would say it was closer to 20 per cent of the patients out of the many thousands that have now been operated upon who had really good results And then Keith Grimson did I think on the basis of Prof Heymans's work the physiologically intelligent procedure of removing the total chain The question then arose in the totally sympathectomized patient is the result worth the effort? Grimson still thinks it is and I should say looking at some of his cases that that might be true But again he was working on an inhomogeneous hypertensive population so he had exactly the same difficulty that all the rest of us had All he could say was that his good results rose to around 40 per cent He is a very careful observer and I accept his conclusions as valid compared with the 20 per cent that the rest of *us were getting with the less extensive sympathectomies* But I think the problem then comes how do you select patients that have a sufficiently high neurogenic element so that you are justified in doing it? Admitted that all patients have some neurogenic tone consequently hexamethonium will give some benefit in most patients But if you really want to get a pressure clear down to normal not just these so called significant falls in blood pressure then I think you've got to find some way of selecting a patient so that you can say that the genetic mechanism involved is really attacked And until we find that we're just going to go on getting statistically significant results without necessarily benefiting the individual patient greatly

von Euler With regard to the question that Dr Page and others have raised the possibility of selection of patients who would be suitable for any kind of blocking agent which would depress the neurogenic element I think perhaps the noradrenaline output in the urine might be an indicator In those cases where it is normal or low I don't think there would be very high activity of the adrenergic system

Platt I don't think you'll ever select the cases which would do well with sympathectomy because the result depends on the anatomical details of the sympathetic nervous system from a surgical point of view and not on neurogenic tone

Goldenberg We started about a year ago to evaluate hypertensive patients undergoing thoracolumbar sympathectomy The two measures found helpful in differentiating cases of essential hypertension were the twenty four hour urinary excretion of noradrenaline and the blood pressure response to *infused noradrenaline* Prof von Euler has just suggested the use of the noradrenaline excretion figure as an indicator of sympathetic tone on the other hand he discredited yesterday the value of the urinary excretion figures stating that they may reflect less the total amounts of noradrenaline produced by the body than the fraction escaping normal metabolic destruction

There are so far twenty cases in our group The distribution of noradrenaline excretion values in this group is similar to that observed in another small group of hypertensives (35 cases) About two thirds were in the normotensive range (up to 41 μg in twenty four hours) one third ranged from 41 to 100 μg This by the way is the highest

excretion figure we have ever found in a case of essential hypertension. These figures are lower than the ones observed by Prof. von Euler. This may be due to the fact that our group is much smaller than his or due to the fact that our patients were on complete bed rest. Since exercise increases the noradrenaline excretion considerably, comparable data can only be obtained if the patients are on complete bed rest.

We too were a bit hesitant to take the amount of noradrenaline excreted in the urine as an index of the total amount of noradrenaline produced in the body. We were therefore pleasantly surprised to find that the patients who excreted large amounts of noradrenaline were quite insensitive to infused noradrenaline as judged by their blood pressure response. This is what you would expect from the concentration action curves. If you add infused noradrenaline to the noradrenaline present at the peripheral sympathetic nerve endings, the blood pressure response will be smaller if larger amounts of noradrenaline have been present prior to the infusion.

The group of hypertensives with noradrenaline excretion figures in the normal range shows an increased sensitivity to noradrenaline, judged by the blood pressure response to infused noradrenaline.

We seem to be dealing here either with two different stages of the same disease or, more likely, with two different types of hypertension, one group showing an increase of sensitivity to noradrenaline, the other (smaller) group an increase of urinary noradrenaline excretion.

So far we have not been able to observe the long range results. The immediate response to sympathectomy seems to be more marked in the group with high noradrenaline excretion.

We have been doing this series in collaboration with Dr. Hinton of New York University.

Genest: Prof. McMichael, what happened to the renal function in the eight patients with malignant hypertension who were treated with C6 and who returned to active life?

McMichael: None of the patients with blood ureas much above 100mg/100ml. survived any length of time, but up to about that level kidney function remained unchanged. If the blood urea was under 100 there was usually no sign of any deterioration.

Genest: That was your only criterion?

McMichael: Yes.

got remarkably good results and some remarkably poor results I would go a little higher than 10 per cent I would say it was closer to 20 per cent of the patients out of the many thousands that have now been operated upon who had really good results And then Keith Grimson did think on the basis of Prof Heymans s work the physiologically intelligent procedure of removing the total chain The question then arose in the totally sympathectomized patient is the result worth the effort Grimson still thinks it is and I should say looking at some of his cases that that might be true But again, he was working on an inhomogeneous hypertensive population, so he had exactly the same difficulty that all the rest of us had All he could say was that his good results rose to around 40 per cent He is a very careful observer and I accept his conclusions as valid compared with the 20 per cent that the rest of us were getting with the less extensive sympathectomies But I think the problem then comes how do you select patients that have a sufficiently high neurogenic element so that you are justified in doing it Admitted that all patients have some neurogenic tone consequently hexamethonium will give some benefit in most patients But if you really want to get a pressure clear down to normal not just these so called significant falls in blood pressure then I think you ve got to find some way of selecting a patient so that you can say that the genetic mechanism involved is really attacked And until we find that we re just going to go on getting statistically significant results without necessarily benefiting the individual patient greatly

VON EULER With regard to the question that Dr Page and others have raised, the possibility of selection of patients who would be suitable for any kind of blocking agent which would depress the neurogenic element I think perhaps the noradrenaline output in the urine might be an indicator In those cases where it is normal or low I don t think there would be very high activity of the adrenergic system

PLATT I don t think you ll ever select the cases which would do well with sympathectomy because the result depends on the anatomical details of the sympathetic nervous system from a surgical point of view and not on neurogenic tone

GOLDENBERG We started about a year ago to evaluate hypertensive patients undergoing thoracolumbar sympathectomy The two measures found helpful in differentiating cases of essential hypertension were the twenty four hour urinary excretion of noradrenaline and the blood pressure response to infused noradrenaline Prof von Euler has just suggested the use of the noradrenaline excretion figure as an indicator of sympathetic tone on the other hand he discredited yesterday the value of the urinary excretion figures stating that they may reflect less the total amounts of noradrenaline produced by the body than the fraction escaping normal metabolic destruction

There are so far twenty cases in our group The distribution of noradrenaline excretion values in this group is similar to that observed in another small group of hypertensives (35 cases) About two-thirds were in the normotensive range (up to 41 μg in twenty four hours) one third ranged from 41 to 100 μg This by the way is the highest

or 17 4 per cent of the material A significant increase was therefore only found in the remaining 16 4 per cent (von Euler Hellner and Purkhold, 1954) The proportion of hypersecretors was fairly constant being 13 17 25 16 and 11 per cent respectively in the five consecutive groups of 100 patients

The significance of this finding is not easy to evaluate however When compared with the excretion figures in phæochromocytoma sometimes up to 3000 μg per day, the excess over normal in the present material is not impressive However the possibility has to be borne in mind that even a moderate hyperproduction of noradrenaline under certain conditions might be a contributing factor in hypertension Goldenberg and his co workers (1948) have found that the pressor response of hypertensive patients to noradrenaline may be considerably increased

Of the total 68 per cent were women and 32 per cent men Within these groups the percentage with noradrenaline excretion above normal was about 36 per cent for females and 30 per cent for males The highest figures (above 144 μg) were more common among women (20) than among men (4)

The distribution in various age groups showed an increasing preponderance of the high figures in the higher ages without any marked difference between the sexes In some patients where paroxysmal blood pressure rises up to 300 mm have occurred the noradrenaline excretion was only moderately elevated This probably excludes the possibility that the attacks have been due to a release of pressor material from chromaffin cell tumours

In a small group of patients with malignant essential hyper tension selected for adrenalectomy some estimations of the noradrenaline excretion before and after adrenalectomy were made The results are given in Table I

The range of excretion of noradrenaline before operation is within the normal range and no consistent alteration is found after adrenalectomy In one of the four patients a marked improvement of the conditions was noted with a fall

CATECHOL AMINE EXCRETION IN URINE IN CASES OF HYPERTENSION

U S VON EULER

AT the Ciba *Foundation Conference on Visceral Circulation* in 1951 I had the opportunity to present the results of catechol amine analysis in urine from 287 cases of hypertension *Differential analyses of adrenaline and noradrenaline were* not made in these cases, only the noradrenaline equivalent of the pressor action of the urine extract on the cat's blood pressure was determined In the majority of cases the differ ence between this figure which includes adrenaline, and the true figure is small, however The present hypertension material which comprises 500 unselected cases, has not been classified in detail, but the great majority of the cases belonged to the group of essential hypertension

From observations on various groups of healthy persons and hospitalized patients with minor disorders and not in bed the average noradrenaline excretion per twenty four hours was estimated at 30 *μg* with a standard deviation of about 15 The upper limit of the normal range thus would be 60 *μg*

The hypertensive patient material has been divided into groups with excretion multiples of *28 8 μg*, corresponding to an average excretion of 20 m*μg* per minute The group excreting *57 6 μg* per twenty four hours would thus correspond fairly well to the upper limit of the normal range Of the total material about 66 per cent had a noradrenaline excretion within the normal range (up to 60 *μg* per day) while in 34 per cent the excretion was above normal In those cases where the excretion rate was 40–60 m*μg* per minute or 57 6–86 4 *μg* per twenty four hours the increase may still be regarded as insignificant This group comprised 87 patients

THE RELATIONSHIP BETWEEN SODIUM ARTERIAL HYPERTENSION AND THE ADRENAL GLANDS

J GENEST

THE work to be reported here which has been carried out for three years (from 1948 to 1951) at the Rockefeller Institute for Medical Research in New York has some interest especially in view of the recent splendid work of the Middlesex Hospital group It was done in order to find out

(1) Whether or not any relation could be detected between the level of the blood pressure in patients with essential hypertension and the adrenal secretion as measured by the urinary steroid excretion

(2) Whether the physiological adaptation of these patients to the stress of severe and prolonged sodium restriction was reflected by any quantitative change in the urinary steroid excretion special attention being given to the corticosteroids

Before presenting the data obtained and discussing the results a word about the method used is necessary

It was realized early in this study that the chemical methods available for the measurement of the various adrenal functions, with particular reference to the regulation of sodium could not yield the desired information We turned instead to a column chromatographic method described by Archibald (1949) which we slightly modified as outlined in Fig 1

The urine is collected for a twenty four hour period at the end of which a 500 ml aliquot is refluxed with 0 1 volume of concentrated HCl for thirty minutes and then extracted with two successive 0 1 volume portions of carbon tetra chloride

The residue of the combined extracts is dissolved in such a volume of benzene that 5 ml of this solution is equivalent to

in blood pressure after a lapse of some months This improvement was not accompanied by any change in the noradrenaline excretion

In one patient (not included in the Table) a peculiar type of urinary excretion was noted in four instances before operation the urinary extract after adsorption on alumina produced a

Table I

NORADRENALINE EXCRETION IN FOUR CASES OF MALIGNANT HYPERTENSION BEFORE AND AFTER ADRENALECTOMY

Patient	Age	Sex	μg noradrenaline per twenty-four hours		
			Before adrenalectomy	After adrenalectomy	
				Unilat	Bilat
N K N	53	M	30–38 **34** (3/5)	—	20–104 **62** (18/322)
N R N	45	M	29–70 **46** (6/7)	15–82 **37** (5/21)	6–91 **46** (14/220)
S S	38	M	44 64 **54** (2/9)	**40** (1/3)	—
B G A	20	M	9 4–51 **27** (6/8)	11–68 **36** (6/16)	**25** (1/9)

Figures in boldface represent averages Figures within brackets denote number of determinations over period of days

strong depressor effect on the cat's blood pressure This patient died two days after adrenalectomy in a shock like state, although all the other patients, including several with operations for malignant tumours stood the operation well The reason for this peculiar reaction pattern and the nature of the atypical excretion product could not be elucidated

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for their 17 ketosteroid content by a modification of the Zimmermann reaction The first, third and fourth fractions gave with the Zimmermann reagent a pink colour typical of the 17 ketosteroids The others gave a light to dark brown colour

Becau e little was known about the composition of the

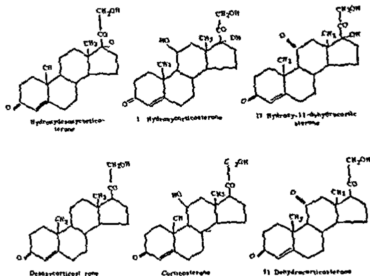


FIG 2 The known corticosteroids which can maintain the life of adrenalectomized rats Note the common Δ^4 unsaturated 3 ketone which is responsible for their ultra violet absorption, maximal at 240 millimicra. These compounds have in addition a ketol side-chain on C_{17} which liberates formaldehyde on oxidation with periodic acid

various fractions obtained by this procedure and because evidence previously available pointed to the known cortico steroids (Fig 2) as the substances most likely to influence the sodium excretion we attempted to find out in which fraction these steroids were eluted by our chromatographic procedure and whether or not they were altered by the procedure used

5 per cent of the daily urinary output A 5 ml portion of this solution is then chromatographed on a column of 2 5 g of grade 3 aluminium oxide *The height of the alumina column* is 70 mm Elution is effected by the use of seven successive solvents of increasing polarity (benzene 20 ml, 0 1 per cent ethanol in benzene 78 ml, 0 5 per cent ethanol in benzene

Chromatographic Fractionation of Urinary Steroid Extracts

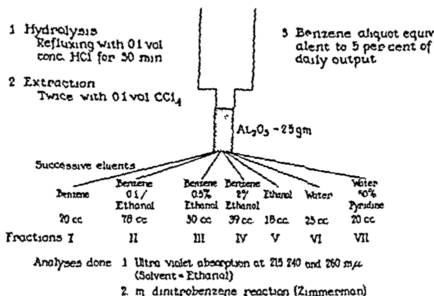


FIG 1 Schematic representation of the procedure used in this study

30 ml 2 per cent ethanol in benzene 39 ml, 95 per cent ethanol 18 ml distilled water 25 ml 50 per cent pyridine in water 20 ml)

These eluents are collected separately *and evaporated to* dryness on a steam bath Each residue is dissolved in 4 ml of distilled 95 per cent ethanol and analysed for its absorption of ultra violet light at wavelengths 215, 240 and 260 milli micra, in a Model DU Beckman quartz spectrophotometer The fractions are then re evaporated to dryness and analysed

extracted with CCl_4 most of the product was likewise eluted in the water fraction although some of the material was lost in the process This material still gave a brownish colour with the Zimmermann reagent The apparent recovery of these corticosteroids after acid refluxing and extraction from aqueous solution and after elution from an aluminium oxide column was on the average 44 per cent in fraction 6 as measured by the ultra violet absorption at 240 millimicra

Since 8 to 9 µg of any one of these corticosteroids dissolved in 4 ml of ethanol have an optical density of 0 100 at 240 millimicra it could be assumed, with reservations that 18 to 20 µg should be easily detected in the fraction eluted with water

The next step was to find out if the eluted material had been qualitatively altered during the procedure This was done in three ways (Genest 1951)

First it was found by Corcoran and Page s method (1948) for the determination of corticosteroids that only 3 to 15 per cent of the initial amount of the untreated corticosteroids could be accounted for as formaldehyde liberated from the water eluates after chromatography on aluminium oxide (Table I) This finding suggested that there had been a *profound* modification of the C_{17} ketol side chain

Secondly *Zaffaroni* (Zaffaroni *et al* 1950) has shown that the addition of concentrated sulphuric acid to a corticosteroid results in a characteristic absorption spectrum and that this procedure has great value in establishing the identity of these compounds We found that the absorption curves of the untreated corticosteroids under the conditions prescribed by Zaffaroni were markedly different from those obtained either after refluxing with 1/10 volume concentrated hydrochloric acid for thirty minutes or after chromatography on aluminium oxide as shown in Figs 3 4 and 5

Thirdly comparison of the infra red spectra (Figs 6 and 7) of cortisone and 17 hydroxy corticosterone acetates with those of the products eluted after adsorption of these cortico steroids on aluminium oxide showed differences in the

When the following untreated corticosteroids (Table I) corticosterone, 11 dehydro corticosterone acetate, 11 deoxy corticosterone, 17 hydroxy corticosterone, cortisone and 17 hydroxy 11 deoxy corticosterone acetate were dissolved in benzene and chromatographed in the same way as the urine

Table I

The figures on the lower left hand side in the columns under U V absorption indicate the total percentage recovery (T R) in all seven fractions whereas those in the centre of the same columns show the percentage recovered in the water eluted fraction The percentile amount of these corticosteroids recovered in the same fraction is indicated in the second column as formaldehyde liberated after periodic acid oxidation This strongly suggests profound modification of the C_{17} side-chain after adsorption of these compounds on MgO

	[illegible] 6			[illegible]
	U V Absorption λ 240 mμ		H_2CO Liberation	U V Absorption λ 240 mμ
C[illegible]on (B) T R	84	80	3	52 — 2
11 Dehydro[illegible]one (A) T R	85	78	3 5	33 — 35
11 De[illegible]y[illegible]on (DOC) T R	86	93		75 — 66
17 O[illegible] D[illegible]y[illegible] (S) T R	73	55	3 5	71 — 32
C[illegible]one (E) T R	53	42	1	50 — 35
[illegible] C[illegible]on (F) T R	4	5	5	42 — 4

CH_2OH–C=O (17) + HIO → H_2CO + COOH (17) + HIO_3

extracts, it was found that the major part of each of these steroids was eluted by water that is they appeared in fraction 6 These eluates gave in each case the same dark brown colour with the Zimmermann reagent as did the compounds before chromatography

If the same corticosteroids dissolved in 2 5 per cent ethanol in water, were separately refluxed with 1/10 volume concentrated HCl prior to the chromatographic procedure and

mental conditions were kept identical the results are still significant

Subjects Seven patients with essential hypertension, two males and five females were studied They ranged from

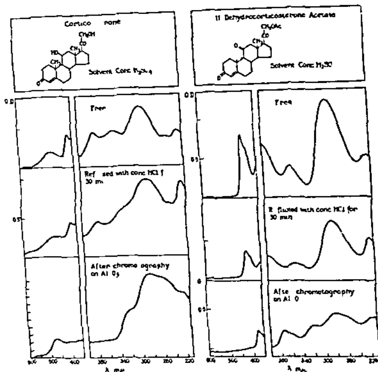


FIG 4 Changes in the absorption spectra determined by the Zaffaroni procedure of corticosterone and of 11-dehydrocorticosterone acetate following acid refluxing or adsorption on Al_2O_3

26 to 44 years of age and were free from any renal involvement Five of the seven patients were admitted to the hospital for six to seven consecutive months and the remaining two for six to eight weeks Their stay in the hospital was divided into approximately two equal periods one on a low sodium

"fingerprint region" (1150 to 800 cm^{-1}) In the carbonyl region (1600 to 1800 cm^{-1}) the spectra of the products of these *two corticosteroids show absorption bands*, but these are not characteristic of 21 acetoxy 20 ketosteroids There

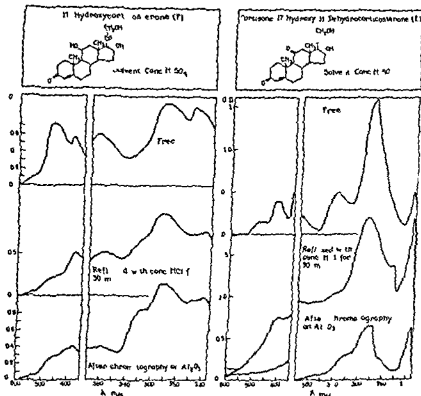


Fig 8 This figure illustrates the profound changes in the absorption spectra determined by the Zaffaroni procedure of 17 hydroxycorticosterone and of 17 hydroxy 11 dehydrocorticosterone following refluxing with concentrated HCl or adsorption *on* Al_2O_3 *These absorption curves* were done two hours after addition of concentrated H_2SO_4 in an automatic recording Carey U V Spectrophotometer

is, therefore, evidence for alteration in the side chain The bands characteristic for the Δ^4 3 ketone system are evident The nature of the eluted products was not studied further

Like everyone else interested in the steroid field, we realize the inadequacies of the methods used But, since the experi

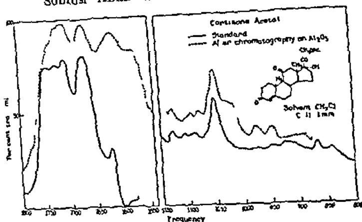


FIG 6

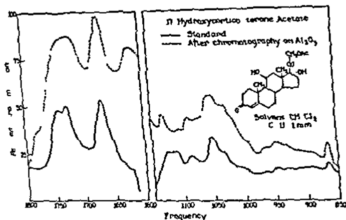


FIG 7

FIGS 6 and 7 The infra red absorption spectra of the acetates of cortisone and of 17 hydroxycorticosterone in what Dobriner has called the "fingerprint region (1150 to 800 cm⁻¹) as well as in the carbonyl region (1800 to 1600 cm⁻¹) show marked differences with those of the products of these compounds eluted by water after adsorption on Al_2O_3 The absorption bands of the C_{17} side-chain are profoundly altered

diet containing 9 milli equivalents of sodium per day and one on high sodium intake, consisting of the same diet supplemented by 174 milli equivalents of sodium given daily as sodium chloride capsules

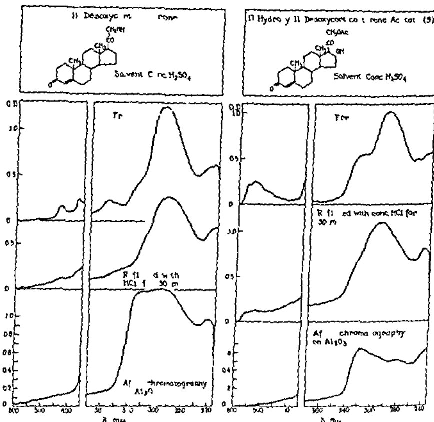


Fig [illegible] Changes in the absorption spectra as determined by the Zaffaroni procedure of 11 desoxycorticosterone and of 17 hydroxy 11 desoxycorticosterone acetate following acid refluxing or adsorption on Al_2O_3

After two to three weeks equilibration during each period two to six separate twenty four hour urine collections were made for steroid fractionation according to the method described above

Results In the seven patients studied the state of adaptation to a severe and prolonged restriction of sodium

difference was apparent in any one fraction obtained from the seven patients studied Fig 9 illustrates the values of the corticosteroid containing fraction ' in four patients two of whom had a lowering of their blood pressure to normal following the low sodium diet

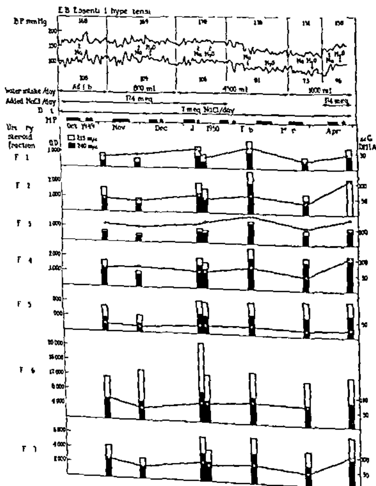


Fig 8

whether or not it is attended by any lowering of the blood pressure to normal, is not accompanied by any consistent modification in our seven urinary steroid fractions as judged by their ultra violet absorption or their Zimmermann reaction values This absence of any correlation is especially striking in fraction 6, which contains the corticosteroids or the products derived from them

The results of the urinary steroid fractions done at various intervals on one patient (E B) are shown in Fig 8 This patient, a 26 year old woman with essential hypertension of three years' duration, was admitted to the hospital for a seven month period during which she received a diet containing 7 to 9 milli equivalents of sodium per day From October 5th 1949 to February 2nd, 1950, and from April 10th to May 1st 1950, she received, in addition 10 g of sodium chloride in capsules It is clear, on the one hand, that the sodium restriction had a definite effect in lowering the blood pressure to normal and on the other hand that there was no significant change in any of the seven fractions studied during the seven month period

The results from the other six patients were similar

The averaged values of the ultra violet light absorption of the seven fractions while each patient was on high sodium intake were compared with the averaged values given by the same fractions while each patient was on a low sodium intake There was considerable overlapping between the individual values of the fractions in a given patient and no significant

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Fig 8 Patient E B —Rockefeller Institute Hospital History No 12479 She was a twenty six year old woman with essential hypertension of three years duration and was admitted to the hospital for a seven month period during which she received a diet containing 7 to 9 milli equivalents of sodium per day From October 5th 1949 to February 2nd 1950 and from April 10th to May 1st 1950 she received in addition 10 g of sodium chloride in capsules It is clear that the sodium restriction had a definite effect in lowering the blood pressure to normal The values of the ultra violet absorption as well as those of the Zimmermann reaction (expressed on the right hand side as micrograms of dehydroisoandrosterone) represent 1/20 of the total daily urinary output The clear dots show the Zimmermann values The height of the clear column is the optical density (O D) at 2[illegible] millimicra and the height of the black column is the O D at 240 millimicra

indicate that the regulation of sodium is not mediated through the known corticosteroids as evidenced by the values obtained in the "corticosteroid containing fraction"

One of the most important contributions in this field is the recent one made by the Middlesex group of Tait, Simpson and Grundy (1952). Their work emphasizes the need to return to the bio assay method for the detection and roughly quantitative determination of the sodium retaining factor, which in a most beautiful and elegant fashion they have shown to be distinct from the actually known active cortico steroids

Much of the confusion and the contradictory results on this subject in the literature come from the fact that the methods employed were not a specific measure of the sodium retaining hormone (or hormones). A difference from the normal values in urinary 17 ketosteroids, reducing lipids, formaldehydogenic or glycogenic substances does not indicate *per se* a modification in the sodium retaining activity of the adrenal cortex

Until the sodium retaining factor (or factors) can be isolated and its (or their) chemical structure identified so that a sensitive and specific method can be devised for its (or their) determination in plasma and urine it will be necessary to use a tedious bio assay method such as the ones described by Simpson and Tait (1952) and by Marcus, Romanoff and Pincus (1952)

This method of estimating the urinary sodium retaining factor(s) will have its greatest value only if combined with a chromatographic separation (Burton *et al* 1951, Bush 1952) of the substances contained in the "crude lipid" extracts of urine. This approach will be our programme for the next three years at the Hotel Dieu Hospital in Montreal

So far the only work we are aware of along this line, is that done by B. Singer (1952) at McGill University. Using the urinary excretion of ^{24}Na in adrenalectomized rats as the basis of her bio assay method Dr Singer found in four patients with essential hypertension under treatment with

Discussion In view of the *strong clinical and experimental evidence* showing a relation between the sodium intake, the adrenal activity and the hypertensive process,

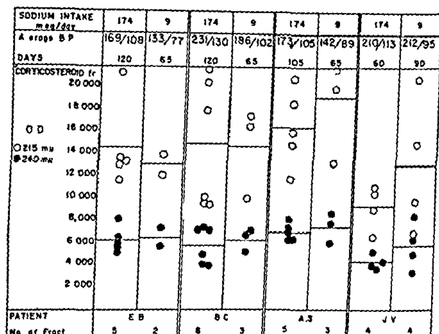


FIG 9 This figure illustrates the effect of variations in sodium intake on the blood pressure of four patients and their corticosteroid containing fraction (expressed as optical density (O D)) at 215 millimicra (O—clear circles) and at 240 millimicra (●—dark dots) The blood pressure is shown as an average for the indicated number of days of the periods during which the patients remained on a high (174 milli equivalents) or on a low (9 milli-equivalents) sodium intake The horizontal bars in each rectangle represent the averages of the values of the O D at wavelengths 215 and 240 millimicra The number of circles and dots corresponds to the number of fractionations done during each period for a given patient Considerable overlapping of the results in the two periods can be clearly seen in the four patients The other three patients show similar results

these negative results could well indicate that the procedure used may not measure the substance presumably of adrenocortical origin, responsible for the sodium conservation and related to the hypertensive process They could also

Haines of the Upjohn Company for some 17 hydroxycorticosterone and cortisone to Dr Percy Julian of the Glidden Company for the 11-deoxy 17 hydroxycorticosterone acetate to Dr Irwin C Winter for the corticosterone to Dr Edward Henderson of the Schering Corporation for the deoxycorticosterone to Dr Geoffrey Rake of the Squibb Company for the 17 hydroxy 11-deoxycorticosterone

We are very grateful to the late Dr Konrad Dobriner and to Dr Estella Katzenellenbogen of the Sloan Kettering Institute of New York for the infra red spectra and for the 17 hydroxycorticosterone used for this analysis

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DISCUSSION

LEDINGHAM Dr Genest if one artificially raises the blood level of cortisone or of other steroids or administers DCA is there a proportionate increase in urinary output? Is the urine output of adrenal steroids a fair indication of their rate of production or is it possible that they are being destroyed by the kidney?

GENEST I must repeat that we do not think our method of measuring steroids is a *true reflection* of the adrenal secretion The urinary extracts contain many other substances besides steroids and it is more than probable that we are not extracting all the steroids contained in the urine In addition the aluminium oxide is responsible for the modifications found in the corticosteroids after their elution Despite these limitations it is possible to detect 18 to 20 μg of modified corticosteroids in the water fraction 6 as measured by ultra violet absorption or by the Zimmermann reaction

low sodium diet or with ion exchange resins, values similar to those obtained from normal subjects These findings were the results of "spot" determinations on each one of the four patients and the assays were done on the "crude" chloroform extracts of acidified urines The possibility of interfering *sodium excreting substances* like renin (Corcoran *et al*, 1951) and cortisone (Davis *et al*, 1951) at the twenty minute dosage level, cannot be ruled out by this technique

The possible existence of sodium retaining factors of non adrenal origin must not be neglected in clinical states such as cardiac failure liver cirrhosis, or the nephrotic syndrome, where the adrenals appear histologically normal The recent work of Lane (1951) and of Schwartz and his co workers (1951), in which hypophysectomized dogs showing atrophic changes in all three adrenocortical layers, responded normally to potassium loads or to sodium restriction gives support to this possibility

Conclusion Seven patients with essential hypertension were studied for two to seven consecutive months under conditions of high sodium and of low sodium intakes Their urinary steroid excretion was determined at frequent intervals according to Archibald s chromatographic fractionation procedure

Restriction in the sodium intake to 9 milli equivalents per day resulted in a fall of the blood pressure to normal levels in five of the seven patients The state of adaptation of the seven patients in response to a severe and prolonged restriction of sodium whether or not there was any change in their blood pressure, was not accompanied by any significant modification in the seven urinary steroid fractions obtained and especially in the "corticosteroid containing fraction

I am very grateful to Drs V P Dole G Cotzias and L K Dahl for permission to use some of their patients and data and for criticism to Drs R M Archibald and D Dziewiatkowski for valuable criticism and advice

We are greatly indebted to Dr J M Carlisle and Dr Augustus Gibson of Merck and Company for the supply of cortisone 17 hydroxy corticosterone and 11 dehydrocorticosterone acetate to Dr Wm J

A STUDY OF ADRENAL INSUFFICIENCY AFTER TREATMENT OF HYPERTENSION BY BILATERAL SYMPATHECTOMY PLUS UNILATERAL ADRENALECTOMY

P ETIENNE MARTIN

I BELIEVE that the secretion of the adrenals is not the usual cause of hypertensive disease in man but that it is an indispensable link in the production and maintenance of this disease which originates elsewhere Total adrenalectomy (Addisonian or surgical) in patients with hypertension gives ample proof of this by the permanent fall in blood pressure which it causes

I wish to show today that operations on the sympathetic system (extensive splanchnicectomy or sympathectomy either alone or preceding unilateral or partial bilateral adrenalectomy) have a temporary more or less prolonged effect by creating adrenal insufficiency (medullar and cortical) which in all clinical probability is largely responsible for the hypotension which results and for the more or less prolonged arrest of the patient s arterial disease This does not in any way prejudge the question of the mode of action of the adrenal secretions in the production of arterial hypertension

Case I A woman aged fifty one was admitted to the Medical Clinic on April 28th 1952 with advanced arterial hypertension with a clinical history of twenty one years It had been discovered during her first pregnancy It recurred during two later pregnancies the last occurring when she was forty years old All three pregnancies ended with the death of the child

Between the ages of forty and forty six years the gradual appearance of severe subjective symptoms was observed with systolic blood pressures in the region of 240 mm Hg

In one instance, we have given 100 mg of DCA in six days to a patient with essential hypertension With our method DCA if excreted as such in the urine, will be *detected in fraction 8 in the proportion of* 68 per cent In this experiment we could not find any significant difference in this fraction from the control level This finding indicates that DCA is metabolized in the body or excreted in a way which made it impossible to determine it by our procedure

LEDINGHAM Is it fair to conclude from these experiments that there is no increase in adrenal steroid production in the hypertensive cases you have studied?

GENEST I would not go that far I would say that our findings constitute presumptive evidence that there is no change in the urinary steroid output in these patients with essential hypertension as determined by our method of analysis

HELMER We have determined the steroids in urine by the Porter Silber method before and after intensive sodium restriction and found no change in excretion

PERERA We can also confirm Dr Genest s observations But there is one factor to be kept in mind namely that rigid salt restriction will cause demonstrable changes in the glomerulosa zone of the adrenal cortex

HELMER I think there are changes in the 17 ketosteroids in rat studies reported in the *American Journal of Physiology* by Danford

PERERA I believe a few have claimed minor changes but the majority of observers have not seen consistent alterations in the excretion of steroids by current methods

LEDINGHAM If adrenal morphology can be taken as evidence of adrenal function then it is appropriate to mention that in experimental renal hypertension, hypertrophy of all zones of the adrenal has been described that is not only of the zona glomerulosa but also of the inner zones of the cortex I wonder whether it would be possible to apply your methods to the small amounts of material from experimental animals

GENEST Since the recent work of Tait Simpson and Grundy we have to start anew in this field We were just searching in the dark and now some light has been thrown but in a rather different direction involving different methods We were searching at a level which was much higher than the one found by Tait and his group for electrocortin which is apparently the sodium retaining hormone and which is present in fractions of a microgram per litre of body fluids

through the diaphragm and resected at the level of the eleventh twelfth dorsal vertebræ Adrenal gland normal Renal biopsy Wound closed with drainage

In the course of the operation the blood pressure fell from 190/190 to 120/80

The post operative course was uneventful the blood pressure rose again to 160/90 in ten days and when the patient got up she noticed that the left paresis had disappeared

On June 12th she returned to the hospital with asthenia which seemed to us greater than usual in patients who have undergone a similar operation

The late result of the operation was good A month after wards the blood pressure was steady at 170/90 Apart from the considerable improvement in the functional symptoms the clinical examination carried out on June 12th showed little appreciable change Cerebral angiosclerosis Disappear ance of subjective signs of arterial hypertension (no headache vertigo or tinnitus) although the blood pressure still varied between 160 and 180 mm Hg Improvement in the neuro logical symptoms The patient walked and spoke normally Retinal angiosclerosis fundus unchanged Cardiac angio sclerosis no change in the orthodiagram or electrocardiogram Renal angiosclerosis excretion tests (P S P and Ambard coefficient) still showed some disturbance but thiosulphate and mannitol clearance were improved

After a month s rest in a convalescent home the patient returned to the medical clinic for the second part of the operation

Examination in September 1952 showed an improvement in general condition a gain in weight of 4 kg and mainten ance of the improvement in the functional symptoms There was no change since the last clinical evaluation except aggravation of the renal involvement Slight albuminuria (10 mg /100 ml) had appeared without casts in the urine The P S P excretion had diminished further and Ambard s coefficient was higher than in June

At forty six years she had a hypertensive attack with symptoms of cerebral œdema (headache, vertigo, vomiting) lasting fifteen days, and at fifty years cardiac decompensation set in during an attack of pneumonia, accompanied by a gallop rhythm, after which there remained considerable dyspnœa after exertion, and slight nocturnal pseudo asthmatic attacks A few months later, left hemiparesis set in gradually and later still slight cerebral eclipses with brief periods of aphasia

Examination on April 28th 1952 showed the blood pressure to be 210/110 grade II sclerosis of the retinal vessels, with moniliform arteries Gunn s phenomenon without exudate or hæmorrhage sclerosis of the cerebral vessels, grade III, with severe arteritis (cerebral attacks with left hemiparesis speech disturbances, headache vertigo, mental sluggishness uncertain memory) cardiac arteriosclerosis grade III dyspnœa on effort and lying down slight malleolar œdema oliguria tachycardia cardiac hypertrophy shown by radiography and a diphasic rapid wave in lead 3 renal angio sclerosis grade II traces of albumin diminution in urea thiosulphate and mannitol clearance

Considering the late pregnancy the earliness of the disturbances during pregnancy and the later development of the disease, I diagnosed essential hypertension with secondary nephrosclerosis and eliminated the true hypertensive nephropathy of pregnancy and the transitory hypertension which recurs during every pregnancy Renal biopsy fully confirmed this diagnosis showing benign nephro angiosclerotic lesions

Rest in bed a low chloride diet and antihistaminic drugs reduced the blood pressure from 220/110 to 160/90

It was then decided that operation was indicated because of the cardiac and cerebral symptoms and to prevent renal sclerosis

First stage May 31st 1952 (Dr J Leclerc) *Right sympathico splanchnicectomy* Closed circuit nitrous oxide ether and oxygen anæsthesia Incision along the twelfth rib and resection of this rib A large splanchnic nerve was easily resected The paravertebral chain was exposed followed

After three weeks this treatment was suspended for two months (January and February 1953) but although the general condition was better and cicatrization made satis factory progress the asthenia and hypotension persisted The patient showed Addisonian pigmentation of the face but there were no mucous patches The hormonal and humoral findings confirmed the adrenal insufficiency disturbance of sugar metabolism (provoked hypoglycæmia and the adrena line test were abnormal) Keppler s test was positive, the blood potassium was 16 mg per 100 ml and the 17 keto-steroids and 11 oxysteroids were diminished

We believe this to be a functional insufficiency of the remaining right adrenal resulting from the sympathectomy Daily intramuscular injections of Syncortyl 5 were given for a few days but did not appreciably alter the clinical picture and in particular had no effect on the blood pressure which remained low On March 10th treatment with cortisone and Stenandiol was begun This was the treatment to which the patient responded best It brought about

(1) A considerable improvement in general condition in appetite and in mental state with an improvement in strength (the patient got up about ten days after treatment was begun) and a gain in weight (10 kg)

(2) A slow fading of the facial pigmentation

(3) Restoration of the blood pressure to normal It had been 100/60 since the operation and rose to 120–130/70–80 in eight days remained at this level for a month then reached 130–140/90–100

(4) Humoral and hormonal effects which are shown in Tables I and II

On May 5th the cortisone treatment was stopped (total dose 2 g orally) but the blood pressure and clinical improve ment were maintained

At present the blood pressure is 130—140/80–90 The pig mentation has disappeared but there remains some physical and mental weakness (subnormal physical activity getting up for meals and for a walk in the grounds in the afternoon)

Second operation, October 8th, 1952 (Dr F Leclerc) *Left adrenalectomy* General anæsthesia *Nesdonal ether, oxygen closed circuit* *Left infra costal incision* The adrenal gland found above the kidney, enveloped in fat, seemed enlarged Dissection was difficult because of the patient's obesity, and a deep vessel, perhaps a lumbar vein, was injured during the operation, causing severe hæmorrhage The adrenal was quickly removed, together with a specimen of kidney for biopsy, and a clamp was left on the bleeding vessel The blood pressure, which could not be measured at the end of the operation, was restored by treatment with pressor drugs, injection of cortin and intravenous fluids, when the patient awoke it was 110/70

The immediate postoperative course was complicated The blood pressure rose satisfactorily with the use of cortin, reaching 180/80 in four days, but a considerable rise of temperature set in, with deep pain in the lumbar fossa on the side operated upon A collection of pus formed, which was only partly cleared by antibiotics

Ten days after the operation typical symptoms of acute adrenal insufficiency set in The general condition became very poor the complexion earthy and there was a rapid loss of weight, temperature 40 , severe asthenia profuse diarrhœa constant vomiting The blood pressure was 100/50, and the heart rate high

The patient returned to the Medical Department on November 23rd, 1952

Until December 1st the diagnosis had been centred on the suppuration, which had been held responsible for the symptoms, but from then on the patient was treated from the adrenal point of view by instituting treatment with cortin pressyl, glucose and saline (in small quantities because of the renal symptoms) and sterandryl This quickly transformed the general condition stopped the vomiting and diarrhœa in eight days, and made feeding possible The lumbar suppuration improved the mass palpated diminished gradually, and the temperature became normal at 37

Table I

HYPERTENSION IN A WOMAN AGED FIFTY-ONE WHO HAD UNDERGONE RIGHT SYMPATHECTOMY AND LEFT SYMPATHICO-ADRENALECTOMY. RENAL AND ADRENAL FUNCTION TESTS OVER A PERIOD OF EIGHT MONTHS

mm Hg	April 52	May 52	June 52	July 52	August	September	October	November
G l C sts	0		0	0		0		few
A buminuria	trac		0	0		10		120
ed B ood Cells	0		0	0		0		very few
Phenolsulphonphthalein Test	70		45			25		
mbard Formula	093		29	142		14		
Ur Clearanc		66	92			88		
stance of Clearanc		78		10		86		
Hyposulf Clearance		85		96		92		
B ood Urea mg %	33	3	36			48		65
Blood Chloride		186/355		198/34		198/320		
B ood Proteins mg				8000				
B ood Cholester l mg %				220		275		
1 et steroid mg/				41				

Table II

OBSERVATIONS IN THE SEVEN MONTHS FOLLOWING TABLE I

	December 1952	Ja uary 53	F bruary 53	March 53	Apr l 5	May 53	J 53
Gra la C st	0	0	0	0		0	0
buminu	25		32	35	55	trac	0
R d Blood C	0	0	0	0			0
Phenolsulphonphthalein Test			38 /	40 /	35	30 /	
Ambar Formula			088	093	164	156	151
U Clearanc			0	73		55 48	
Blood Ur g /	34		5 40	0 0	35 45	55	
Blood Chloride	195/355		/3 8	m/ 56	195/ 5	00 16	
Blood Protein mg			6800			5750	6400
Ser Sodium mg				350			
mg Blood Sugar mg			78 83	90	85	88	3 5
Insulin Tolerance Test mg			53-62-68				
Blood Cholesterol mg %			225		214	2 0	2 0
Serum Pot ssium mg			16		20 8	18 8	2 3
7 etos eroid mg %	35				67		47
0-Oxyst roid g %	0 8		00 7			0	09
Thorn Test			61 %				
Keppler in			Adrenal insufficiency		Adrenal ins ncy	Normal ncy	

Investigation of the hypertensive disease shows complete disappearance of the cerebral disturbances, headaches, vertigo and tinnitus Speech is normal without stumbling The patient walks normally without dragging the left leg as she did before the operation *There are no more nocturnal attacks of pseudo asthma, nothing remains but slight dyspnoea* after climbing stairs The electrocardiogram is almost unchanged It is noteworthy that the low voltage of the deflections has not been appreciably changed by the adrenal treatment

A chart (Tables I and II) recapitulating the tests carried out in this case makes it possible to follow the course of the hypertensive *disease* and of the *adrenal insufficiency in this* patient

Pathological examination of the adrenal and of the kidneys (Prof Guichard and Dr Cabanne) gave the following results

1 *Adrenal Gland*

Capsule fibrous and thickened with *slight* arteriolitis and hyalinization of the wall of a few arterioles Glomerular zone *partly disappeared showing a typical glandular struc*ture at only one point Spongiocytic zone diffuse and slight hyperplasia, with a syncytial picture here and there Reticular zone normal Medullary tissue clearly visible normal A single lymphocytic islet with 5–10 cells was found in the reticular zone

2 *Kidneys*

The lesions are *the* same in both fragments *Diffuse* lesions of benign nephro angiosclerosis

Such cases of severe adrenal insufficiency of Addisonian type are unusual after sympathico adrenalectomy Besides the one just described I have seen two others almost identical

Case 2 This was in a man of thirty one years with juvenile arteritis, whom we sent for *splanchnicectomy* with right adrenalectomy in one stage followed by partial *left* adrenalectomy in a second stage The Addisonian state with intense blackish, slaty pigmentation of the forehead root of the nose dorsum of the hands and forearms *and a blood pressure of*

Addison's syndrome due to and maintained by functional inhibition of the denervated left adrenal which remained

Humoral and hormonal tests confirmed the adrenal insufficiency (low blood sodium high blood potassium intolerance to insulin good tolerance of adrenaline)

Leriche and later Weiss have each reported a case of death after operation on the splanchnic nerve and one adrenal which they attribute to an endocrine origin (probably acute adrenal insufficiency)

But apart from these severe cases of adrenal insufficiency it seems to me now that neuro endocrine operations bring about sub clinical adrenal insufficiencies remaining obscure through lack of revealing factors On the other hand in some cases like the one I have just reported an adjuvant factor in the form of suppuration brings to light the hidden adrenal insufficiency and transforms it into a major Addisonian insufficiency

From this arises a two fold physio pathological problem Does denervation of the adrenals by sympathectomy alter the cortical and medullary secretions in the direction of under activity? Do sympathectomy and partial adrenalectomy act on hypertensive disease mainly by diminishing the cortical and medullary secretions of the adrenals?

Does denervation of the adrenals by sympathectomy alter the cortical and medullary secretions?

Physiological ideas on this subject have changed greatly during the past twenty years in a first period Sgrosso and then Hermann showed in dogs that denervation of the adrenal does not produce any histological change in the gland which preserves its characteristic function i e the production of adrenaline Fontaine Froehlich and Mendel came to the same conclusion though emphasizing the fact that denervation suppresses reflex adrenaline secretion As for the adrenal cortex the anatomical and histological studies of Galloni Fontaine Fontanini Fernando de Castro, Potar and MacFarland proved that its innervation which comes from the

90/50 obliged me to institute treatment with cortisone and to *implant 600 mg of DCA and 700 mg of testosterone*, which soon diminished the pigmentation, weakness and hypotension, but led to a fresh attack of arteritis

Case 3 A man of fifty six years, who had had severe hypertension since the age of forty nine Stage III (severe) cardio angiosclerosis was the indication for left *sympathico* splanchnicectomy as a first stage and right splanchnicectomy and adrenalectomy as a second stage operation

Pathological examination (Prof Guichard)

Adrenal Thickening of the capsule Very numerous thickened arterioles, with hyalinization only at one point in the section Glomerular zone absent in three quarters of the specimen, sclerosis of the glomerular zone in places Very *great thickening of the spongiocytic zone, reticular zone* normal, generalized hyperplasia of the medulla Very numerous lymphoid islets disseminated in the cortex and medulla

The *Kidney* showed nephro angiosclerotic lesions stage II (hyalinization without infarction), and perfectly intact glomeruli

Adrenal insufficiency, with weakness, fatigability after effort, and *a low blood pressure (about 115/85)* set in very rapidly after the second operation

Treatment with cortin rapidly improved the patient, who went home to Italy before we had had time to make a complete study of the course of his adrenal insufficiency

In 1936 Rogoff described the appearance of Addison's disease after bilateral adrenal denervation for diabetes mellitus

In 1948 Campina published the case of a woman with disturbances from high blood pressure and hyperthyroidism in whom the hypertensive syndrome was replaced, after operation on the adrenals (right adrenalectomy and left splanchnicectomy), by a new complex of symptoms which, although there was no cutaneous pigmentation or hypotension suggested to the author the hypothesis of partial

vascular anastomoses at the cortico medullary boundary consisting of simple communicating canals with fibro endothelial walls and only glandular cells around their borders Thanks to these vascular anastomoses, which develop from the age of three years passage through the vessels is possible in all directions that is to say, from the cortical arteries to the central medullary veins and from the central veins of the medulla into the peripheral veins of the cortex Changes in the lumen of these vascular channels are probably determined by contraction of the pericytes which contract under the influence of adrenaline

The contraction of the pericytes can allow or block the common cortico medullary circulation in other words it can allow the cortical blood to flood the medullary cells and the medullary blood to flood the cortical cells or it may force the arterial blood of each gland in the adrenal to flow into its own veins

Leulier and Revol have shown histo chemically that the medulla is as rich in cholesterol as the cortex and Gineste has discovered that ascorbic acid which is known to be plentiful in the cortex has a very distinct medullotropic action This intimate anatomical relation cannot exist without a nervous and secretory relationship

The nervous factor was studied by Poznan in 1938 with Hilarowicz and Kubikowski He carried out denervation of the adrenal by division of the splanchnic nerves partial excision of the semilunar ganglia and division of the anastomoses between the adrenals and the sympathetic system He observed histological changes in the gland characterized by vacuolization in the cortical and medullary systems The chromaffin cells showed less affinity to salts of chromic acid These disturbances which appeared seven days after denervation became more and more marked and then gradually disappeared eight to ten months later

Weidenmann has recently reported (1952) his observations on the effect of lumbar sympathectomy on the testicles and adrenal cortex of the cat He showed that the adrenal cortex

splanchnic and dorso lumbar nerves, only provides it with vasomotor, and not with secretory fibres

For a long time, therefore, we were influenced by the belief that adrenal denervation had only a moderate effect on the medulla and none at all on the cortex

However, relevant researches undertaken during the last few years are beginning to modify this opinion Though nothing has appeared to supplement Hermann's original research on the adrenal medulla, histological and experimental work shows us that the adrenal cortex is not, as had been thought, independent of denervation or of the medullary secretion By studying neurite degeneration after division of the splanchnic and dorso lumbar nerves, Kiss showed that the adrenal medulla receives only preganglionic fibres, and that it is, in fact in accordance with Elliott s idea the equivalent of a sympathetic ganglion On the other hand the cortex receives the postganglionic fibres which are generally distributed in the blood vessels their termination in cells being only very rarely seen, as Corte Real observed

And yet, whereas embryological, histological chemical and physiological ideas had led to a dualistic conception of these two glands opposing any interaction or functional synergism, recent experiments are opposing this dogma Da Costa recalls the intimate anatomical relation between these two parts of the gland in mammals The juxtaposition of the two glands is effected by genuine interlocking the cortical end of a medullary cord lies between two medullary ends of cortical cords and the medullary end of a cortical cord lies between two cortical ends of medullary cords This arrangement is maintained with perfectly regular alternation Along each cortical and medullary cord there is an arteriole and a venule which form capillaries and anastomose around the cells Da Costa says it would be contrary to all the evidence to suppose that this intimate connexion which is even closer than that between the thyroid and parathyroids has no significance or consequence In support of this morphological argument, Velican has just described a system of

and ascorbic acid content of the adrenal cortex In 1946 J Malméjac who extended this study to dogs rabbits and guinea pigs reached the same conclusions This author took the diminution in the number of lymphocytes in the blood stream as the test for hypersecretion His experiments fully confirmed Marthe Vogt s findings

The question naturally arose whether adrenaline either injected or secreted by the medulla acts directly or through the pituitary Marthe Vogt believes the action to be direct because it persists after hypophysectomy Morros Sarda thinks the same and Vlichean goes so far as to admit the possibility of cortico medullary regulation by a neuro endocrine mechanism through the intermediary of a colloidal substance travelling along the nerves but Long and Malméjac believe that the action is indirect They have seen the effects of adrenaline on the cortex abolished after hypophysectomy and in Sayers s opinion adrenaline acts on the pituitary either directly on the glandular cells or through the intermediary of the hypothalamus which in turn stimulates the pituitary gland Hume and Wittenstein think that adrenaline brings about the intervention of a hypothalamic product which stimulates the production of corticotrophin in the anterior pituitary and that this causes the overactivity of the adrenal cortex However this may be the activity of the adrenal medulla is faithfully echoed by that of the cortex All these experiments show clearly that the functional links between the two parts of the adrenal are very subtle and constant when the glands are in their normal condition with their innervation intact

The secretion of the adrenal medulla is governed by the preganglionic cholinergic fibres of the sympathetic nervous system Consequently the sympathetic system can act on the adrenal cortex through the intermediary of the adrenal medulla adrenaline serving as a chemical mediator between the vegetative nervous fibres and the hypophysio cortical system (Malméjac)

Though all these physiological findings and experimen

of the cat is divided into three clearly differentiated zones glomerular, fasciculate, and reticular, and that the medullary tissue, whose limits are clearly marked, is divided into separate lobules The lesions following sympathectomy reach a maximum after two months The adrenal cortex as a whole acquires a homogeneous appearance The different zones lose their own character The changes are most marked in the middle of the fasciculate zone, whose breadth is diminished Pyknotic lesions are observed in the reticular zone, adjacent to the medulla The lobular structure disappears in the medulla, while the chromaffin cells, having shrunk, are no larger than the chromophobe cells Six months after the operation the changes within the adrenal are well on the way to retrogression

It seems therefore, that denervation of the adrenal produces profound histological changes in the medulla and in the cortex But the effect of these histological changes on the secretion of corticosteroids has not yet been studied Though both the cortical and medullary parts of the gland seem to depend on the nervous system, a secretory interaction seems to make the cortex dependent upon the medulla either directly or indirectly through the hypophysis

Marthe Vogt in 1944 produced immediate and intense hypersecretion of the adrenal cortex lasting one or two hours, by splanchnic stimulation or by intravenous injections of adrenaline in dogs and cats The cortical overactivity is recognized in the blood of the same adrenal gland by means of Selye and Schenker s method Young adrenalectomized rats treated with cortical extracts are exposed to low temperatures The response i e the duration of survival is found to be proportional to the logarithm of the dose injected The hypersecretion obtained by Marthe Vogt is considerable being four to five times the normal It therefore seems to be proved that in spite of the absence of a secretory innervation of the cortical cells the cortex does respond to nervous stimuli by hypersecretion In 1945 Long and Fry found in rats that injections of adrenaline lower the cholesterol

If therefore extensive sympathectomy changes the histological structure of the adrenal thus diminishing its secretion we must suppose the immediate hypotension which follows operations on the nerves to be due to the adrenal change and that if the beneficial effect lasts only a few months this is because the original activity of the adrenal returns in eight to twelve months as the recent experiments of Weidenmann have shown

But it also appears that partial adrenalectomy adds a notable effect to simple sympathectomy We have shown by our operative results that sympathico adrenalectomy is superior to extensive sympathectomy This also explains the fact that simple bilateral splanchnicectomy sometimes produces results which in the long run are as brilliant as those of extensive sympathectomy

We see at once where the shoe pinches in this surgical treatment of hypertension by acting on an intermediate link in the hypertensive cycle instead of on the original link we produce total adrenal deficiency with the organic and vital losses which it entails in order to stop the secretion of a single corticosteroid of the DCA or some other type produced by a diseased adrenal As soon as this insufficiency is over come the disease reappears though in an attenuated form

Pure sympathectomy and sympathico adrenalectomy are useful methods of treatment in arterial hypertension but the advantage is so dearly bought that we must work per sistently to discover the primary cause of the disease and by this means its proper treatment

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tend to prove in a tempting manner the close relations between the splanchnic nerve and the adrenal gland both medullary and cortical, they do not yet enable us to assert that splanchnicectomy necessarily causes a diminution in the function of these two parts, but they do lead us to suspect it strongly This is a question for the future and its solution is the task of the physiologists

The clinical observations which we have just reported are distinctly in favour of this hypothesis

Do sympathectomy and adrenalectomy act on hypertensive disease mainly by diminishing the medullary and cortical secretions of the adrenal?

It is far from my intention to neglect the effect of sympathectomy on the splanchnic vascular bed, which it dilates by disconnecting it from the vasomotor centres It is also far from my thoughts to overlook the effect of operation on the arterial walls whose enzymatic secretions are altered, according to Jiménez Diaz, also on the walls of the carotid, where the receptors of the regulators of blood pressure are situated, indicating a modified resistance to distension in Volhard s and Heymans' sense, or, finally on the renal artery, in which the flow is increased according to Talbott all these facts have their due value, but I obstinately hold to the constant fact that whatever method may be used to produce hypertension, and whatever the etiology of the human hypertension which is being considered, bilateral adrenalectomy always reduces the blood pressure instantly to a very low level for months

The clinical experiences of Green, Thorn, and Page seem to show that this hypertensive role is played by the mineralo corticoid substance(s) of the DCA type This substance, which may not, properly speaking be an adrenal hormone is capable by itself of producing malignant arterial hyper tension (in the presence of large quantities of sodium chloride, a condition which, by the way can rarely occur in disease)

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DISCUSSION

PICKERING Prof Etienne Martin in the patients you have described did you extirpate the adrenal at the first operation?

ETIENNE MARTIN No at the second

PICKERING And you often had the Addisonian pigmentation after removal of one adrenal only?

ETIENNE MARTIN In three cases of unilateral adrenalectomy with sympathectomy of the other side

PICKERING And the adrenal on the other side was normal?

ETIENNE MARTIN Yes

GOVAERTS What happens to the secretion of adrenaline and of corticoids after section of the sympathetic nerves innervating the adrenals? According to Stewart and Rogoff after cutting the sympathetic nerve going to the adrenal you do not get any more secretion of adrenaline I don t know whether that is still accepted by physiologists

HEYMANS The sympathetic innervation is certainly important for the normal function of the medullary part of the suprarenal gland I think we need more information concerning the influence of the sympathetic innervation on the cortical suprarenal function

PERERA There are no demonstrable changes in chromatographic fractionation of urinary steroids after experimental sympathectomy Similarly we have not seen any consistent changes in the adrenal glands

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PERERA G A (1945) *J Amer med Ass* 129 537

de Lyon 1948) and Guichard and Cabanne (in *La Revue Médicale du Moyen-Orient* 1952) have described lesions in the adrenals of hypertensives Much of this work was done with adrenals which I have removed from my patients

These authors have drawn up a histological picture of the lesions to be expected in the adrenal in hypertensive disease the commonest findings are the presence of lymphocytic islets (73 per cent) and muscular hyperplasia of the central adrenal vein (73 per cent) next in frequency spongiocytic hyperplasia (69 per cent) and finally there should also be noted the fibrosis of the capsule with arteriolitis and hyalinization (63 per cent) as well as either loss or fibrosis of the zona glomerulosa 59 per cent)

themselves histologically So I wonder whether this patient might not have had the coincidental development of Addison s disease at some point close to the time of the second procedure when one suprarenal gland was removed

ETIENNE MARTIN We have made observations in three cases of adrenal insufficiency In each of them we have begun with a unilateral sympathectomy at the same time noting the condition of the kidney and the adrenal Later we have removed the adrenal on the opposite side Two were cases of hypertension and the third was a case of Buerger s disease

I recently asked Prof Stahl of the University of Strasbourg his opinion on the subject and he said that he has observed the same Addisonian pigmentation in a patient on whom he had just performed a splanchnicectomy and adrenalectomy

I think that these cases are more frequent than we think We have never sufficiently investigated the disorders of slight adrenal insufficiency because we have always maintained that sympathectomy and splanchnicectomy have no effect on the adrenal as the physiologists have always instructed us Probably there must be exceptional cases where there are factors as in the cases I have just reported which will transform these latent adrenal insufficiencies into severe adrenal insufficiency that is into true Addison s disease

VON EULER Concerning the secretion of medullary hormones after denervation or splanchnicectomy Marthe Vogt has done such experiments There is a certain resting secretion but it is definitely lower than what one finds in the normal anaesthetized animal in the suprarenal venous blood Such an animal of course doesn t respond to hypoglycemia or reflex stimuli

PAGE Isn t it true that chemical stimulation with histamine and other substances even in the denervated gland causes secretion of adrenaline and noradrenaline?

VON EULER Yes

PAGE So that you can have a continued secretion regardless of the innervation of the gland?

VON EULER Yes similar to the secretion going on from denervated salivary glands

PAGE Are there sympathetic ganglia in the medulla that respond to chemical stimulation? A strong sympathetic ganglion stimulator like dimethylphenylpiperazinium iodide or nicotine may stimulate the ganglia *in situ* which starts secretion of pressor amines Histamine has a relatively weak stimulating action on the adrenal medulla compared with DMPP

GENEST Prof Etienne Martin in your printed summary you made a statement which surprises me to the effect that the *usual* lesions of hypertensive adrenalitis are disappearance of the glomerulosa hyperplasia of the fasciculata normal reticularis normal medulla and moderate arteriolitis How specific are these findings for arterial hypertension and how often do you see these lesions in this disease?

ETIENNE MARTIN Guichard and Boulliat (in the *Journal de Médecine*

hours after bilateral nephrectomy there was in our rats a definite increase in the extracellular fluid volume (thiocyanate or inulin volume) and (2) that a close correlation existed between the accumulation of water and salt in the extracellular spaces and the associated hypertension

In 1949 Grollman Muirhead and Vanatta produced hypertension in bilaterally nephrectomized dogs maintained alive for several weeks by peritoneal lavage Although Grollman s collaborators (Muirhead Fogelman, *Jones and Graham* 1953) still insist that hypertension can occur in dogs after bilateral nephrectomy in the absence of an exogenous Na excess and in the absence of an expansion of extracellular fluid volume the work of Leonards and Heisler (1952) and Orbison Christian and Peters (1952) *seems to show* that in this condition there frequently exists a fluid and Na retention and a definite increase in extracellular fluid volume The former found that the inulin space was doubled in the animals which developed hypertension following overloading with fluid while no hypertension developed *in those maintained in* fluid balance The latter showed that *hypertension* developed rapidly and consistently in dogs within twenty four *to forty* eight hours after bilateral nephrectomy when 100 *ml per kg* per day of isotonic NaCl or *electrolyte solution* was injected intraperitoneally Houck (1953) confirms these findings showing that if *overhydration is* prevented nephrectomized dogs do not develop hypertension and that the increase in blood pressure is always associated with an increase in extracellular fluid volume

The above mentioned results *may* also throw light on the results obtained by those who have studied the course followed by the blood pressure after total nephrectomy in animals with *previous* renal experimental hypertension Thus Ogden (1947) in rats and Pickering (1945) in rabbits observed that after bilateral nephrectomy the blood pressure fell more or less rapidly in animals with short standing renal experimental hypertension but in animals in which hypertension had been present *for a relatively long period of time*

WATER AND ELECTROLYTES IN EXPERIMENTAL HYPERTENSION

E BRAUN MENENDEZ

SINCE we found that *hypertension after bilateral nephrectomy* in the rat (Braun Menendez and von Euler, 1947) was *associated with an increase in blood volume and extracellular* fluid volume (Braun Menendez and Covián, 1948) we became interested in the study of the possible relation between salt and water metabolism and experimental hypertension In a previous review (Braun Menendez 1951) I have given an account of our investigations and a review of the literature In these last years many new data have accumulated which confirm and extend our work and constitute important evidence in favour of the participation of disturbances in water and electrolyte metabolism in the genesis of experimental hypertension I believe, therefore, that an exposition of the findings of ourselves and others in this respect is timely in order to facilitate a tentative synthesis and the subsequent discussion

We shall discuss three apparently quite different types of experimental hypertension hypertension following total nephrectomy nephrogenic hypertension and deoxycorticosterone hypertension

Hypertension following Bilateral Nephrectomy

The effect of bilateral nephrectomy on the arterial pressure has been the *object of numerous investigations* The results obtained were contradictory and it was generally accepted that the removal of both kidneys did not produce hypertension until in 1947, we reported (Braun Menendez and von *Euler 1947) the occurrence of hypertension in bilaterally* nephrectomized rats We also showed, shortly afterwards *(Braun Menendez and Covián 1948) (1)* that forty eight

may well find its solution if we admit—in the light of the work mentioned—that a disturbance in the water and electrolyte concentration of the extracellular and intracellular fluids may cause overstretching with lysis and rupture of the medial muscle fibres of the arterioles followed by repair

The intravenous (Winternitz *et al* 1940) or subcutaneous (Masson *et al* 1953) administration of renin into totally nephrectomized dogs also elicits a syndrome of accelerated malignant hypertensive vascular disease especially if 1 per cent NaCl solution is given as drinking fluid There is ample evidence that renin causes changes in electrolyte distribution and cellular permeability, and its action on the muscle fibres may be due to a change in the distribution of electrolytes in and around cells thus altering their permeability to proteins and ultimately causing their death (Masson *et al* 1953)

Renal Hypertension

Hypertension produced in rats by unilateral perinephritis is associated with a significant increase in extracellular fluid volume as Braun Menendez and Martinez (1949*a*) have shown This has been recently confirmed by Grollman and Shapiro (1953) in the dog

Eichelberger (1943) has observed in renal hypertensive dogs an increase in extracellular fluid with abnormal distribution of electrolytes She demonstrated an increased sodium and chloride and a decreased potassium content of muscle in the hypertensive as compared to the normal Laramore and Grollman (1950) confirmed these findings in hypertensive rats Greene and Sapirstein (1952) also found that total body sodium is considerably increased in rats made hypertensive by subtotal nephrectomy

The water and salt metabolism in experimental renal hypertension is disturbed even in the absence of recognizable renal insufficiency In rats made hypertensive by unilateral perinephritis the other kidney being left intact there is polydipsia and polyuria (Chanutin and Ferris 1932 Oster and Martinez 1943 Braun Menendez 1950*a* Green *et al*

the blood pressure remained high until death supervened As we shall see later, in experimental renal hypertension a disturbance in water and electrolyte metabolism exists, characterized by increased extracellular fluid volume and retention and maldistribution of sodium This pre existing abnormality, similar to that caused by bilateral nephrectomy, very probably enhances the effects of the latter, thus keeping the blood pressure at a high level Floyer (1951) showed that the fall in blood pressure of renal hypertensive rats after nephrectomy and adrenalectomy could be prevented by *giving 1 per cent saline as drinking fluid* *The greater increase* in blood pressure after bilateral nephrectomy of rats pre treated with deoxycorticosterone (Friedman and Friedman, 1949 Hall and Hall 1949) may also be explained by the same mechanism The results presented here strongly suggest that hypertension after bilateral nephrectomy is closely linked with fluid and electrolyte retention and, as we shall see later, presumably with some disturbance in the electrolyte concentration of extracellular and intracellular fluids

Bilateral nephrectomy is also followed in the dog by arteriolar necrosis and subsequent fibrosis and by myocardial lesions which show great resemblance to the lesions of malignant hypertension (Winternitz *et al*, 1940 Holman, 1943, Grollman *et al* 1949 Muirhead *et al*, 1953, and others) The appearance and incidence of these lesions are accelerated and incremented by the occurrence of hypertension (Muirhead *et al*, 1953) or fluid and salt overloading (Orbison, Christian and Peters, 1952) in the dog, and by high salt diet in the rat (Masson Corcoran and Page, 1951*b* Tobian 1950) Turner and Grollman (1951) suggest that the vascular lesions described may appear as a consequence of failure of a *mechanism controlling the ratio of sodium and potassium* between the cells and the interstitial fluid The old controversy as to whether hypertension alone (Wilson and Pickering, 1938 Wilson and Byrom 1939 Byrom and Dodson 1949) is or is not (Goldblatt 1951) a sufficient condition for the production of experimental sclerosis and arteriolar necrosis

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1953) *Stamler and Minimi (1952) observed the same* during incipient nephrogenic hypertension in dogs Hyper tensive rats are also more liable to dehydration than normal rats, during total fasting they excrete in twenty four hours a urine volume nearly three times as great as that of normal rats (Braun Menendez, 1950*a*) We also showed that after administration by stomach tube of saline in amounts corres ponding to 5 per cent of body weight, the hypertensive animals eliminate in the next twenty four hours a greater percentage of water and of NaCl than the controls This was interpreted by us as due to an excess of Na in the organism of the hypertensive animal, which, therefore, rejects addi tional salt more readily Green, Sturtevant and Van Arman (1953) have confirmed this finding during the early stage of renal hypertension This phenomenon can be related to the aversion for sodium that hypertensive rats show when given the choice of different salt solutions (Abrams *et al*, 1949 *Tosteson et al*, 1951) *They drink one third to half as much* NaCl and $NaHCO_3$ as the normal rats

An increased rate of urinary excretion of antidiuretic *substance has been found in rats and dogs with experimental* hypertension (Ellis and Grollman, 1949) If the increased *renal excretion of antidiuretic principle really means an* increased production of antidiuretic hormone by the posterior *lobe of the pituitary, it may be assumed that this increased* production is a compensatory response to some endocrine or metabolic alteration which requires a retention of water in order to maintain the tonicity of the body fluids The experi ments of Skahen and Green (1948) are in favour of this inter pretation They found that the urinary elimination of the antidiuretic principle is proportional to the water turnover When the fluid intake increases following the administration of deoxycorticosterone or the substitution of water by an 0 8 per cent sodium chloride solution as drinking fluid the elimination of the antidiuretic principle also increases

I will not enter into a thorough discussion of the effect of salt loading and salt depletion on the blood pressure of animals

with experimental hypertension The studies of Grollman and Harrison (1945) Handler and Bernheim (1950) Danford and co workers (1950) and Danford and Herrin (1952) show that increasing or decreasing the sodium intake in rats with experimental renal hypertension causes parallel changes in blood pressure Hypertensive dogs seem to be less sensitive to high or low salt diets (Page and Lewis 1949 McGuire and Wilhelmj 1951) nevertheless a severe chronic salt depletion with hyponatræmia reduced the blood pressure of renal hypertensive dogs to normal (Frieden *et al* 1952)

The effect of sodium overloading on the vascular pathology of renal hypertensive animals has not been studied But the intravenous injection of renin into renal hypertensive dogs precipitates cardio vascular lesions typical of malignant hypertension (Leiter and Eichelberger 1942 1943) and the subcutaneous injection of renin in rats pre treated with deoxycorticosterone and hypertonic salt solution causes hæmorrhages œdema and diffuse vascular lesions (Masson Corcoran and Page 1951*a*)

Deoxycorticosterone Hypertension

Following the administration of deoxycorticosterone to rats dogs and men there is striking and rapid reduction in the renal excretion of sodium and chloride and an increased renal excretion of potassium The retention of sodium is accompanied by an enhanced appetite for sodium (Braun Menendez 1950*b* Braun Menendez and Brandt 1952) a marked and rapid increase in the extracellular fluid volume (Braun Menendez and Martinez 1949*a* Gaudino and Levitt 1949 Davis Bass and Overman 1951) a decrease in intra cellular fluid volume (Gaudino and Levitt 1949) and a slight increase in blood volume

Investigations on the electrolyte content of various tissues and organs show that potassium leaves the cells and is replaced by sodium This change has been observed in voluntary muscle and also in cardiac muscle (for literature see Overman 1951)

The prolonged administration of deoxycorticosterone causes in rats renal lesions polyuria and hypertension This action is strictly dependent on the presence of sodium in the diet or the drinking fluid In the absence of sodium the administration of deoxycorticosterone is not followed by renal lesions or hypertension or an increased fluid turnover The action of deoxycorticosterone is so closely related to the presence of sodium in the diet or the drinking fluid that it is difficult to decide whether sodium sensitizes the animals to the action of deoxycorticosterone or whether deoxycorticosterone "potentiates the effect of subthreshold doses of sodium chloride" as Selye and Stone expressed in 1943 The latter is true at least in the chick, where the administration of sodium chloride in high concentration (2 per cent as drinking fluid) causes renal lesions (Selye 1943) and hypertension (Lenel et al, 1948), the former being identical with those observed when subthreshold doses of sodium chloride (0 2 per cent) which in themselves are not toxic, are administered in conjunction with small doses of deoxycorticosterone (Selye and Stone 1943)

Sapirstein Brandt and Drury (1950) administered for six weeks 2 per cent saline solution as a drinking fluid to rats The animals developed hypertension associated with heart and kidney hypertrophy Meneely and co workers (1952) and Auerbach and co workers (1953) fed large quantities of sodium chloride to rats for many months and observed œdema, marked increase in extracellular fluid volume, renal lesions and hypertension Hartroft and Hartroft (1952) observed degranulation of renal juxtaglomerular cells in rats given a salt rich diet or deoxycorticosterone Adrenalectomy or a salt poor diet produced on the contrary an increase in granules Gepts (1952) finds necrotic lesions in the myocardium and pancreas and periarteritis in rats overloaded with salt for twelve to forty two days

No significant elevation of blood pressure was observed in normal dogs given a 2 per cent NaCl solution as sole source of drinking fluid (Wilhelmj et al 1951) This is probably due

to the fact that the kidneys of normal dogs have a great ability to eliminate NaCl as shown by Ladd and Raisz (1949)

The cardiovascular lesions produced by the administration of deoxycorticosterone plus salt are similar to those in experimental malignant hypertension The injection of renin into rats pre treated with deoxycorticosterone and excess sodium chloride elicits a syndrome of oliguria œdema hyper tension and convulsions which bears great resemblance to toxæmia of pregnancy (Masson *et al* 1951*a*) At autopsy vascular lesions were found which included widespread visceral hæmorrhages arterial occlusion and renal glomerular degener ation Normal rats showed no ill effects from the injection of similar amounts of renin (Masson *et al* 1952)

All the factors which are known to favour the retention and (or) maldistribution of sodium in the organism facilitate the production of deoxycorticosterone hypertension or if this is present cause an aggravation of the animal s condition Among such factors we may mention unilateral nephrectomy (Selye and Pentz 1943) nephrotoxic serum nephritis (Knowlton *et al* 1946) experimental perinephritis (Braun Menendez and Martinez 1949*b* Freed *et al*, 1951) adminis tration of renin (Masson *et al* 1951a) and experimental diabetes (Braun Menendez and Martínez 1949*b*) Doses of deoxycorticosterone which are insufficient to cause hyper tension in normal animals do cause an increase in arterial pressure in the above mentioned conditions

Conclusions

In this review I have mentioned three different experi mental conditions leading to arterial hypertension and cardiovascular lesions similar to those of the malignant type of hypertension The three conditions have a common denomi nator retention and maldistribution of sodium and water In all three there is an increase in extracellular fluid altered electrolyte concentration of the extracellular and intracellular fluids and disturbances in salt and water metabolism in all three overloading with salt and water accentuates and

accelerates the appearance of hypertension and cardiovascular lesions while adrenalectomy and salt depletion have an inverse effect, in all three, injections of renin which cause serious disturbances in fluid and electrolyte distribution and capillary and cellular permeability precipitate the vascular pathological syndrome

The order of precedence of the "biochemical lesion", as Vanatta (1951) puts it, is difficult to establish and is perhaps different in the three conditions mentioned Retention of sodium may precede its entrance into the cells or vice versa retention of sodium may precede retention of water or vice versa, etc There are some indications pointing to a primary alteration of the permeability to cations following deoxycorticosterone administration Further studies should clarify this problem

It is interesting to speculate as to the mechanism of hypertension and vascular lesions Hypertension, even in the case of post nephrectomy hypertension is not due to plethora and increased minute volume but to an increased peripheral resistance caused by vasoconstriction Apart from the presence in the blood of vasoconstrictor substances of renal, medullo adrenal or other origins, the tone of the muscle cells of the cardiovascular system may be influenced by the electrolyte concentration of intracellular and extracellular fluids and by the state of hydration of the organism The kidney, the adrenal cortex and probably also the posterior lobe of the pituitary should play their roles in this respect According to some investigators the elevated blood pressure alone is a sufficient condition for the production of the cardiovascular lesions which characterize malignant hypertension According to Goldblatt (1938) the combination of increased vascular tension and the effect of a chemical substance of renal origin are necessary conditions for the production of arteriolar necrosis and the associated hæmorrhages" I believe that the results which I have reviewed leave no doubt as to the role of high blood pressure and renin but to these two factors a third should be added which alone

or in combination with the other two may cause similar lesions, and that is the electrolyte concentration of intracellular and extracellular fluids and the state of hydration

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[Discussion of this paper was postponed until after the paper by Dr Ledingham —ED]

THE DISTRIBUTION OF FLUID AND ELECTROLYTES IN EXPERIMENTAL HYPERTENSION

J M LEDINGHAM

IN view of the many similarities between the various forms of experimental hypertension, both in regard to the influence of the intake of sodium chloride upon them, and in regard to the many reported disturbances of water and electrolyte distribution, experiments were planned to investigate the possibility that some underlying disturbance in water and electrolyte distribution is common to all

The experiments to be described deal with the direct measurement of the partition of water and of sodium and potassium between intra and extracellular compartments of heart and skeletal muscle in various forms of experimental hypertension in rats and in particular in experimental renal hypertension (Ledingham 1953) Total body and tissue extracellular fluid were measured by inulin (Ross and Mokotoff 1951), after equilibration had been obtained in animals whose renal pedicles had been ligated Plasma and tissue electrolytes were measured by flame photometry In the calculation of the tissue extracellular fluid volume and its content of sodium corrections were applied for the inequality of inulin and sodium in interstitial fluid and plasma

Experimental Renal Hypertension

A group of 11 rats were made hypertensive by the application of a silver clip to one renal artery the contralateral kidney being left untouched or removed After hypertension had been present for periods ranging from nine to eighty eight days, the group was compared with a group of 11 control rats of similar age which had been subjected to the same treatment save for the application of the clip The duration of

hypertension in the first group and the blood pressure level and extracellular fluid volume per 100 g. body weight in both groups are shown in Table I. Although the mean extracellular fluid volume was higher in the hypertensive group,

Table I

The blood pressure and the extracellular fluid volume per 100 g. of body weight in renal hypertensive and normotensive control rats, the former being arranged in order of increasing duration of hypertension.

Group	N	*Duration of hypertension (days)*	*Blood pressure (mm. Hg.)*	*E.C.F.V. per 100 g. body wt (ml.)*
Hypertensive	H1	9	225	28.9
	H2	13	205	2[illegible].1
	H3	14.5	193	28.5
	H4	16	210	24.7
	H5	30	205	23.8
	H6	32	230	23.4
	H7	37.5	240	25.0
	H8	58	215	20.2
	H9	72	215	21.9
	H10	72	205	21.4
	H11	88	200	20.6
	MEAN	40.2	213.2	23.7
Control Normotensive	N1	—	120	19.7
	N2	—	115	18.8
	N3	—	125	20.7
	N4	—	125	21.2
	N5	—	115	19.5
	N6	—	120	21.1
	N7	—	115	19.8
	N8	—	120	19.2
	N9	—	120	18.2
	N10	—	115	19.6
	N11	—	115	18.9
	MEAN	—	118.6	19.7

there was a fall to the normal range as the duration of hypertension increased. This correlation is shown in Fig. 1, where the horizontal lines are drawn at twice the standard deviation of the extracellular fluid volume per 100 g. body weight of the normotensive controls above and below their mean value

That there is a significant correlation between the extracellular fluid volume per 100 g body weight and the extracellular fluid volume per 100 g of heart muscle is shown in Fig 2

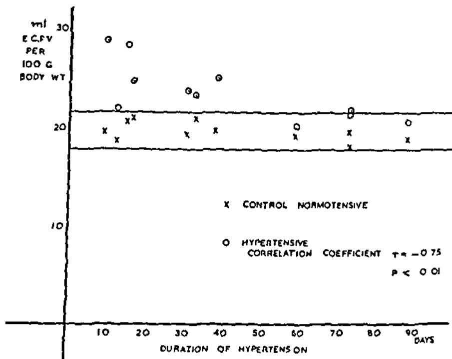


FIG 1 To show the fall in the extracellular fluid volume per 100 g of body weight to within the normal range as the duration of experimental renal hypertension increases Each observation on a hypertensive animal is paired with an observation on a normotensive control The horizontal lines are drawn at twice the standard deviation of the extracellular fluid volume per 100 g of body weight of the normotensive controls above and below their mean value

The mean values for the distribution of sodium and potassium in heart and skeletal muscle are shown in Table II and Fig 3 There was no significant difference between the levels of plasma sodium and potassium in the two groups The mean total sodium and potassium per kg of heart muscle in the hypertensive groups were significantly higher and lower

respectively than in the controls Similar observations have been made by Laramore and Grollman (1950) However these differences decreased as the duration of hypertension increased and appeared to be due to the transient increase in tissue extracellular fluid associated with the increase in body

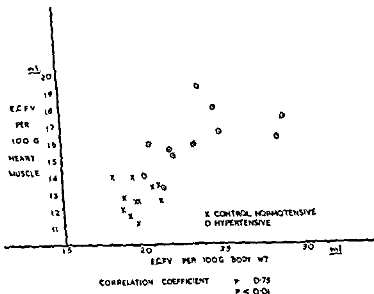


FIG 2 To show the relationship between the extracellular fluid volume per 100 g of heart muscle and the extracellular fluid volume per 100 g of body weight in the combined group of renal hypertensive and normotensive control rats

extracellular fluid The mean concentration of sodium in the intracellular fluid of heart muscle was estimated to be 6 per cent less in the hypertensive group than in the controls but this difference was not quite statistically significant (the 95 per cent fiducial limits were −13 3 per cent and + 1 2 per cent) The intracellular fluid concentration of potassium was the same in both groups In the case of skeletal muscle there was no difference between total sodium and potassium in

Table II

The distribution of sodium and potassium in plasma and heart and skeletal muscle in renal hypertensive and normotensive rats The mean values are given together with the standard errors The ratio of intracellular fluid volume to solids is considered as a measure of the degree of intracellular hydration

			Hypertensive group		*Normotensive group*		*Significance of difference of means*
Plasma	Na	mEq/l	144 2	±1 1	143 9	±0 6	
	K	mEq/l	4 13	±0 11	4 18	±0 17	
Heart muscle	Total Na	mEq/Kg	33 5	±0 7	30 8	±0 4	t=11 6 P<0 01
	Total K	mEq/Kg	80 8	±0 8	93 1	±0 6	t=3 15 P<0 01
	Na Concn in I C F	mEq/l	22 2	±0 6	23 7	±0 6	t=1 78 0 05<P<0 1
	K concn in I C F	mEq/l	147 2	±1 1	148 0	±1 0	
	Ratio I C F V solids	l/Kg	2 45	±0 02	2 42	±0 02	
Skeletal muscle	Total Na	mEq/Kg	18 8	±0 6	18 6	±0 7	
	Total K	mEq/Kg	116 9	±0 5	118 1	±1 1	
	Na concn in I C F	mEq/l	12 2	±0 7	14 0	±0 7	t=1 83 0 05<P<0 1
	K concn in I C F	mEq/l	172 5	±0 9	172 2	±0 9	
	Ratio I C F V solids	l/Kg	2 70	±0 05	2 82	±0 04	

the two groups however the concentration of sodium in the intracellular fluid was 12 per cent less in the hypertensive than in the control group this difference again being not

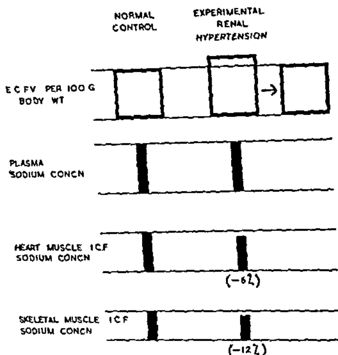


FIG 3 Diagrammatic representation of the findings in a group of rats with experimental renal hypertension and in a normotensive control group Differences from the control group are expressed as a percentage of the latter Figures in brackets indicate that they do not differ significantly from zero

quite significant (the 95 per cent fiducial limits were −27 3 per cent and +2 4 per cent)

Discussion on Experimental Renal Hypertension

An increase in body extracellular fluid volume has been reported by others in experimental renal hypertension (Braun Menendez and Martinez 1949 Grollman and Shapiro 1953)

In the series reported here an increase in body extracellular fluid volume per 100 g body weight has been observed in hypertension of short duration, but this increase above the normal range of extracellular fluid disappeared as the duration of hypertension increased An increase in the extracellular fluid volume is therefore not an essential for the established state of experimental renal hypertension The cause of the increase in extracellular fluid volume found in hypertension of short duration has been further considered It is not simply due to inequality of the weights of the animals in the two groups however, it is possible that at least part of the increase may be due to the loss of body fat which sometimes occurs when the blood pressure is rising acutely, for it is known that in man when the amount of body fat is reduced the extracellular fluid volume per 100 g body weight is increased (McCance and Widdowson 1951, Odier and Mach, 1949) For various reasons the increase is more likely to be due to a temporary interference with the renal circulation brought about by the various operative procedures A transient increase in extracellular fluid volume has been produced by disturbances in the renal circulation not resulting in hypertension It is suggested that any technique for the production of experimental renal hypertension which prevents the development of compensatory renal hypertrophy may be associated with a permanent elevation in the extracellular fluid volume

The small observed decrease in the concentration of sodium in the intracellular fluid of heart and skeletal muscle in experimental renal hypertension, though not quite significant, is of great interest In the calculation of intracellular sodium, the assumed concentration of inulin and sodium in interstitial fluid has been weighted slightly in the direction of narrowing the differences between the two groups Hence it is quite possible that the decrease in the intracellular concentration of sodium is really rather greater and may well be significant

The suggestion has been made (Tobian and Binion, 1952) that experimental renal hypertension may be due to narrowing

of the arterioles brought about by a widespread swelling of muscle tissue In the present experiments using heart and skeletal muscle there is no evidence of an increase in tissue extracellular fluid in hypertension of long duration nor is there any evidence of a significant state of cellular over hydration if a measure of this be taken as the ratio of the intracellular fluid to the weight of the total solids in tissue (see Table II)

Hypertension Produced by Adrenal Steroids

I should now like to refer briefly to some preliminary studies on the distribution of water and electrolytes in other forms of experimental hypertension The first group deals with the hypertension produced by adrenal steroids Small groups of rats were adrenalectomized and treated with deoxycortico sterone acetate (4 × 25 mg implants on day of adrenalectomy) cortisone acetate (1 mg twice daily by subcutaneous injec tion) or a combination of the two The rats were maintained on a diet containing 1 per cent of sodium chloride and drank either tap water or 1 5 per cent saline After varying intervals they were sacrificed and compared with a group of adrenal ectomized rats maintained on 1 5 per cent saline and with normal controls The details of these experiments and some of the findings are shown in Fig 4 Only in the group treated with deoxycorticosterone and cortisone was overt oedema present, and the results here are open to the objection of failure of equilibration The final mean blood pressures were those to be expected from earlier work i e the absence of salt hypertension in adrenalectomized rats the potentiation of deoxycorticosterone hypertension by sodium chloride and the additive actions of deoxycorticosterone and cortisone There was a significant fall in the extracellular fluid volume in the group receiving cortisone in all other groups an increase took place Plasma sodium rose only in the groups receiving deoxycorticosterone and saline As regards the sodium con centration in the intracellular fluid it was remarkable how very variable this was in skeletal muscle compared with the

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with an increase of the extracellular relative to the intracellular concentration of sodium in heart muscle and that between these two the increase of extracellular sodium is the more important

Renoprival Hypertension

The second group of experiments deals with similar measurements in rats three days after nephrectomy with or

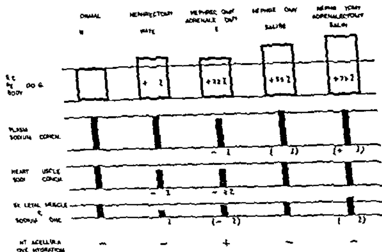


FIG 5 Diagrammatic representation of the findings in a group of normal rats and in groups of rats three days after nephrectomy with or without accompanying adrenalectomy The groups drank either water or 0 5 per cent saline Differences from the normal group are expressed as a percentage of the latter Figures in brackets indicate that they do not differ significantly from zero

without accompanying adrenalectomy The animals were placed on a fat glucose and starch diet for two days prior to nephrectomy and throughout the post operative period They drank either tap water or 0 5 per cent saline Some of the results are shown in Fig 5 There was a wide variation within each group The only group becoming hypertensive was the nephrectomized group drinking 0 5 per cent saline

relative constancy in heart muscle, in fact in the group treated with deoxycorticosterone and saline there was a threefold increase in the intracellular concentration of sodium in skeletal muscle and only a comparatively slight increase in heart muscle There is no apparent common factor in the distribution of sodium between extra and intracellular fluid in heart and skeletal muscle in the groups in which hypertension

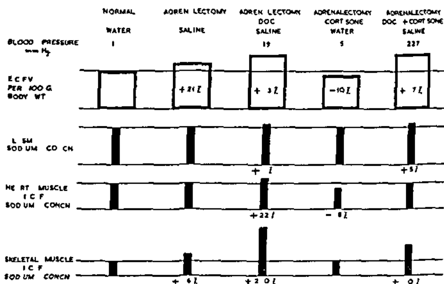


FIG 4 Diagrammatic representation of the findings in a group of normal rats and in groups of adrenalectomized rats some of which were treated with adrenal steroids The groups drank either water or 1 5 per cent saline The percentage figures express the differences from the normal group

was present However, if heart muscle alone is considered in the deoxycorticosterone saline and deoxycorticosterone cortisone saline groups there was an increase in extracellular sodium whereas the intracellular concentration of sodium was raised in the former and normal in the latter In the deoxycorticosterone water and cortisone water groups there was a normal level of extracellular sodium and reduced intracellular sodium in heart muscle These findings are compatible with the hypothesis that hypertension is associated

there has been no evidence of a rise in the level of the intra cellular sodium concentration in heart muscle

Thus it would appear that the adrenals in the absence of kidneys maintain the level of plasma sodium at the expense of sodium in skeletal muscle and to a lesser extent in heart muscle and also prevent hydration of the intracellular com partment These findings are again compatible with the hypothesis that a rise in blood pressure is associated with an increase of the extracellular relative to the intracellular sodium concentration in heart muscle which in renoprival hypertension can be brought about by the adrenal in the absence of kidneys or by sodium chloride in the absence of both kidneys and adrenals

Conclusion

Thus in the three forms of experimental hypertension studied renal adrenal steroid and renoprival although no certain conclusions can be reached at present there is the distinct possibility of some common underlying disturbance of sodium distribution in heart muscle Whether this disturbance occurs in the smooth muscle of the arterial wall and there plays any role in producing vasoconstriction is as yet completely unknown The existence of a disturbance of water or potassium distribution common to these forms of hypertension has been considered but none has been found

Finally it should be said that errors arising in the method of measurement of tissue extracellular fluid have been care fully examined and it is considered that they are unlikely materially to alter the above conclusions

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BRAUN MENENDEZ F and MARTINEZ C (1949) *Rev Soc argent Biol* 25 168
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GROLLMAN A and SHAPIRO A P (1953) *J clin Invest* 32 312
LARAMORE D C and GROLLMAN A (1950) *Amer J Physiol* 161 278

Had a longer time elapsed after nephrectomy, more conclusive differences in blood pressure might have emerged From earlier work of Floyer (1951) it might be expected that the blood pressure in the nephrectomized group drinking water would have risen after this period of three days that the blood pressure *in the nephrectomized adrenalectomized group* drinking water would have fallen further, and that the blood pressure in the nephrectomized adrenalectomized group drinking saline would have risen to the normal level or even become hypertensive In the figure intracellular hydration is estimated as the ratio of intracellular fluid volume to total solids the normal ratio being defined as 100 per cent In both the nephrectomized and the nephrectomized adrenal ectomized groups drinking water there was an expansion of the extracellular fluid volume even though the former group lost weight This observation was made previously by Braun Menendez and Covián (1948) In the nephrectomized group the plasma sodium concentration was fairly well main tained, apparently as a result of a significant withdrawal of sodium from skeletal muscle and possibly from other tissues there was only slight withdrawal from heart muscle The nephrectomized adrenalectomized group tended to gain weight, and the retained water passed into the intracellular compartment in heart and skeletal muscle causing significant intracellular overhydration with lowering of the intracellular concentration of sodium and potassium Sodium was not withdrawn from skeletal muscle to maintain the sodium con centration in the expanded extracellular space In the two *groups drinking* saline there was *an even greater* expansion of the extracellular volume in both groups the plasma sodium concentration was raised slightly but not significantly In the nephrectomized adrenalectomized group there was now *only slight entry of water into the intracellular compartment* but there did appear to have been entry of sodium into skeletal muscle with an increase in the intracellular concen tration of sodium This entry of sodium into skeletal muscle has not occurred *in the case of heart muscle and in all groups*

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DISCUSSION

HELLER Dr Braun Menendez I suppose you are not suggesting that expansion of the extracellular fluid volume and sodium retention is a primary factor in the genesis of hypertension? It may perhaps in certain circumstances be an adjuvant effect The proof of that is quite simple—one can mention any number of clinical cases with expanded extracellular fluid volume and sodium retention and no rise of blood pressure

BRAUN MENENDEZ No I never stated that increased extracellular fluid volume is a cause of hypertension but only an accompanying fact

HELLER But you do suggest that if in a state of hypertension the extracellular fluid is expanded and sodium retained then that could have an influence on the disease?

BRAUN MENENDEZ I think so

PAGE You mean on the lesions of the disease?

BRAUN MENENDEZ On the height of the blood pressure and probably on the lesions

WILSON I wonder if anyone else has observed this reversal of the change in extracellular fluid volume as the duration of hypertension continues I think it most important to have further evidence on that point

GROLLMAN In the human in whom the disease has been present for some time with the development of severe hypertension the extracellular fluid volume is increased This would exclude the possibility suggested by Dr Ledingham that the phenomenon may be the result of surgical interference The question of the effect of loss of weight which he has mentioned is of great importance since the apparent extracellular fluid volume expressed in terms of the body weight is closely related to the amount of adipose tissue present in the body Consequently an apparent increase in extracellular fluid volume may reflect only a loss of adipose tissue rather than a true increase in this volume concomitant with an increase in the severity or duration of the hypertension In considering apparent changes in extracellular fluid volume expressed in terms of the body weight one must take into consideration any changes in the total amount of adipose tissue present in the body

LEDINGHAM Yes I think that does apply certainly in the earliest stages of experimental renal hypertension animals are often very sick and lose weight I have however observed in rats after a severe operation not followed by hypertension the animals may lose their fat but they do not show expansion of the extracellular fluid expressed as a percentage of body weight Thus is rather against the suggested

explanation but on the other hand the loss of weight of these animals is not so severe as it is in some of the animals who are acutely hypertensive that is in hypertension of short duration I think this explanation of the expansion of extracellular fluid volume in short duration hypertension does remain a possibility In long duration hypertension I feel strongly that the observation of a normal extracellular fluid volume is valid the animals by this time have regained their lost fat and are the same weight as the controls

GOVAERTS In the dog when you give large amounts of DCA—which presumably increases the extracellular fluid volume and anyway makes the blood pressure go up —for six weeks and then stop it the blood pressure comes down

BRAUN MENENDEZ That doesn t happen in normal dogs?

GOVAERTS Maybe not always but certainly in some dogs *if you give* large enough amounts of DCA and the pressure goes down when you stop it Of course it is easier to get that hypertensive action of DCA when you have previously removed one kidney or put a clamp on the one kidney

PAGE How high is the blood pressure?

GOVAERTS Fairly high 3 4 cm. Hg higher than normal We had a dog in which we could reproduce that hypertension very easily The dog had one renal artery constricted for a *very long time* but his blood pressure *was normal* The blood pressure went up quite soon and stayed up as long as we gave DCA and then came down if we stopped it after three to four weeks

BRAUN MENENDEZ It wasn t quite a normal dog then?

GOVAERTS No but I mention that particular dog because *his* reaction to DCA was clear cut and the experiment was repeated several times

JIMENEZ DIAZ In experiments that we have reported in the past we found in nephrectomized dogs the same effects seen by Dr Braun Menendez *The extracellular* fluids increased until death But on the other hand we have observed (reported by Castro-Mendoza Merchante and Linazasoro) that *the sodium* content of the plasma remains normal at the same time as *the chloride* is decreasing There is then a true dissociation of the two ions after nephrectomy Have you seen that too Dr Ledingham?

LEDINGHAM I didn t measure chlorides but it is possible that if your dogs were drinking water and they had an expanded extracellular space chloride ions would not be available to maintain the concentration whereas in the case of sodium about 50 per cent or perhaps a little less is intracellular in skeletal and heart muscle and therefore *if* expansion of the extracellular space occurs sodium is available and may be withdrawn from the cells to maintain the level of plasma sodium

JIMENEZ DIAZ This dissociation is the same as various authors have observed clinically in cardiac patients on a low salt diet and mercurial diuretics who were already insensitive to the mercurial diuretics

HELMER We have some evidence that the rate of exchange of sodium in the gastrointestinal tract of patients with hypertension may be different from that in normals We treated seven patients with hyper

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normal? I do not believe that we should put too much emphasis on minor alterations in electrolyte and water metabolism and attribute to them some necessarily fundamental significance They may reflect only a change in the total body economy For example we know that adrenalectomy results in a marked loss of sodium, which soon leads to the death of the animal However if the adrenals are removed in a nephrectomized animal this loss is prevented and the striking effects of hyponatremia do not result The experimental techniques of different observers may lead to varying results due to technical differences in their procedures For example adrenalectomy in the hypertensive animal results in a loss of salt and water with a decline in blood pressure However if the animal is supplied with sufficient salt and water the operation may be done without inducing this decline in blood pressure We can account for the apparent effect of adrenalectomy on the blood pressure solely on the basis of the well established loss of salt and water without assuming that the adrenal plays a specific rôle in the process

I would also take exception to the inference made by Dr Braun Menendez regarding the significance of the increase in extracellular fluid and blood volume observed after nephrectomy Such animals are thirsty and consume large quantities of water It is to be expected that they should become over hydrated which might result in an elevation in their blood pressure However such an elevation in blood pressure should be attributed to the artificial conditions under which the animals are being maintained If one prevents their taking an excess of water and salt and carries out the experiment as Dr Floyer and Dr Ledingham have done one will not observe this increase in extracellular fluid volume nor the increase in blood pressure which Dr Braun Menendez has reported and to which he attaches such significance One should attribute this rise in pressure to the abnormal condition of his experiment and consider it simply as an incidental finding

LEDINGHAM I think I must interrupt here The nephrectomized group receiving saline had a considerable expansion of extracellular fluid volume as all nephrectomized animals do but they didn t gain in weight at all It is these preparations which develop hypertension The adrenalectomized nephrectomized group receiving water also showed expansion of extracellular fluid volume and gained more weight but they did not become hypertensive in fact their blood pressure fell

GROLLMAN Precisely! Since the rat is unable to vomit if it be given access to salt water only overexpansion of its extracellular flui l volume is inevitable Even if not given access to food the breakdown of endogenous protein and the taking of water will result in the same phenomenon These conditions which characterized the experiments of Dr Braun Menendez and Prof von Euler are abnormal and do not tell us what the normal reaction to nephrectomy per se would be If one prevents such an abnormal expansion of extracellular fluid volume by maintaining the animal following nephrectomy on a salt free diet and administers sufficient carbohydrate and fat to depress endogenous protein catabolism, no elevation in blood pressure follows immediately

tension and two normal subjects with a 500 mg diet and 18 g of ion exchange resins daily In normotensive individuals the resin took up 0 5 mEq per gram of resin in hypertensive patients 0 7–1 5 mEq After one month of therapy there was no change in the resin uptake of sodium in the normotensive subjects whereas the resin output of sodium in the hypertensive patients was reduced to 0 5 mEq the same as with normal subjects

BRAUN MENENDEZ I should like to answer Dr Ledingham's question about the duration of hypertension In our experiments we only used rats with long standing hypertension and these had an increased extracellular fluid volume With DCA on the contrary you only find an increase in extracellular fluid volume at the beginning

LEDINGHAM These animals had been given DCA for a month and the hypertension was therefore not of very long duration

FLOYER How long standing Dr Braun Menendez was the hypertension of your rats?

BRAUN MENENDEZ Three to five months

FLOYER One kidney with perinephric fibrosis and the other kidney removed?

BRAUN MENENDEZ The other kidney intact

WILSON It seems to me that there are three particular lines on which we might try to clarify our ideas First is there in hypertension any consistent change in electrolyte distribution? Dr Ledingham's results in various kinds of experimental hypertension differ from those of certain other authors I think it would be of great value if we could exchange ideas on this subject leaving aside for the moment the rather different problem of the abnormal response of the hypertensive subject to administered salt or DCA Secondly I think we might consider whether the work on adrenalectomy and substitution therapy has led to any valid conclusions on the role of the adrenal cortex in experimental renal hypertension and post nephrectomy hypertension Thirdly I should like to hear the views of one or two of our members on the question of hypertension produced by cortical steroids I think there is some evidence that in experimental hypertension we are dealing not with increased adrenocortical activity but with normal adrenocortical activity which is not being controlled I wonder whether by dividing the discussion into these three sections we might perhaps get a clearer idea of the renal adrenocortical relationship in hypertension

PICKERING You suggest that we start by considering what the evidence is for an abnormal distribution of sodium between extracellular and intracellular fluids I take it that the crux of the matter here is that when you have a bit of tissue and you determine its sodium and potassium content how can you determine how much of that sodium and potassium is intra and how much extracellular? Dr Ledingham's answer is that you do it with inulin

GROLLMAN No one questions the fact that there are many differences in the electrolyte and water metabolism of the hypertensive as compared to the normal The question arises as to their significance Do they reflect some fundamental difference between the hypertensive and the

blood pressure of animals after nephrectomy when sodium loss is impossible In Fig 2 the dots represent the blood pressure of a series of rats with chronic renal hypertension which after a control period were subjected to nephrectomy The blood pressure remained raised The circles represent rats with comparable hypertension which were subjected to nephrectomy and adrenalectomy at once the blood pressure fell to and remained at normal or subnormal levels Both groups were given tap water to drink after the operation so that gain or loss of sodium could not occur The fall in blood pressure was not due to poor

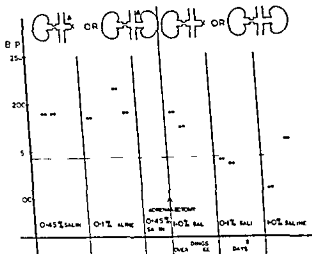


FIG 1 (Floyer) Effect of reduction of salt intake in rats with chronic renal hypertension before and after total adrenalectomy (*Clin Sci* 1951 10 405)

condition of the adrenalectomized rats the duration of life after operation was nearly the same in the two groups I measured the plasma volume of some of these rats this was increased both after nephrectomy and after nephrectomy and adrenalectomy showing that the changes in blood pressure cannot be explained by changes in plasma volume Dr Ledingham has just shown that the extracellular fluid volume increases both after nephrectomy alone and after nephrectomy and adrenalectomy

Fig 3 shows the effect of giving 1 per cent saline instead of tap water after nephrectomy and adrenalectomy The circles represent the rats given tap water after nephrectomy and adrenalectomy which are the same as in Fig 2 the dots show a group of hypertensive rats subjected to nephrectomy and adrenalectomy but given 1 per cent saline after

after nephrectomy It is observed only some days later I consider the latter as true hypertension

BRAUN MENENDEZ There must be some intake of water and salt because you have to give peritoneal lavage to keep your animals alive

GROLLMAN One can elicit the same rise in blood pressure and other effects without resorting to peritoneal lavage or other artificial measures by maintaining the animal on an electrolyte and protein free diet and administering a volume of water equal to that lost in the insensible perspiration

BRAUN MENENDEZ You have to give them food

GROLLMAN They receive only carbohydrate which of course is completely metabolized They receive no protein or salt but merely a sufficient amount of water (25ml /kg of body weight) to compensate for the insensible perspiration In other words the animals are maintained in a normal condition insofar as the salt and water content of the body is concerned and are not allowed to acquire the artificial expansion of the extracellular fluid to which I have attributed the effects which you have observed

LEDINGHAM In the first group of animals shown which were nephrectomized only and were on an electrolyte free diet drinking water the blood pressure admittedly was normal but they were examined three days post operatively Dr Floyer carried his observations on longer and there is no doubt that these animals become hypertensive

PICKERING Did you want to show slides Dr Floyer? There seems to be a discrepancy between your views and Dr Grollman s over the effects of adrenalectomy in the nephrectomized animal

FLOYER Perhaps these figures will help to explain the discrepancy between my results and those of Dr Grollman Fig 1 shows the result of adrenalectomy in seven animals with hypertension Hypertensive animals in which one adrenal had previously been removed were used the first three columns represent a control period before adrenalectomy The rats were on a low sodium diet and it can be seen that reducing the concentration of salt in the drinking water from 0 45 to 0 1 per cent had no effect on the blood pressure After the second adrenal had been removed the animals were maintained on 1 per cent saline it can be seen that the blood pressure fell somewhat but remained at hypertensive levels A week later the salt was reduced to 0 1 per cent resulting in a steady fall in blood pressure which rose again to previous levels when 1 per cent salt was substituted This shows that renal hypertension can be maintained in the absence of the adrenal provided sufficient salt is given but the blood pressure falls when salt is reduced This fall in blood pressure might be due to a fall in plasma volume due to loss of salt and water from the kidney or to the fact that the mechanism which maintains hypertension can only operate in the absence of the adrenal when sufficient sodium is given on the strength of this experiment alone it appears that the first explanation is the more likely I did however notice that the blood pressure fell before there was a significant drop in weight

The next step was to investigate the effect of adrenalectomy on the

operation. In these animals the hypertension persisted after nephrectomy and adrenalectomy There was no constant weight increase from oedema and plasma volume estimation showed a rise comparable to the rats drinking tap water

It can be seen that the adrenal plays a part in the mechanism which maintains hypertension and that the fall in blood pressure after adrenalectomy is not solely due to excessive loss of salt and water through the kidney The finding that giving salt restores hypertension

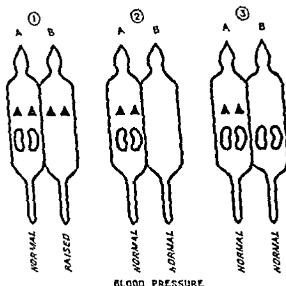


FIG. 4 (Ledingham) Effect of (1) nephrectomy ( ) nephrectomy and adrenalectomy and (3) adrenalectomy alone on the blood pressure of *one member* of a parabiotic pair of rats (Reprinted from Wilson, C 1953 *Lancet* 265 632)

after nephrectomy and adrenalectomy may indicate that the extra renal pressor mechanism is closely linked with the regulation of internal distribution of sodium Dr Ledingham has just shown the results of further investigation into this hypothesis

WILSON I think Dr Ledingham's parabiotic experiments strongly support the view that the adrenals are in fact necessary

LEDINGHAM I should like to give our interpretation of certain experiments which have a bearing on this problem They are concerned with renoprival hypertension occurring *in* parabiotic rats and the results are illustrated diagramatically in Fig 4 If the kidneys are removed from one member of a pair of parabiotic rats (first pair in the figure)

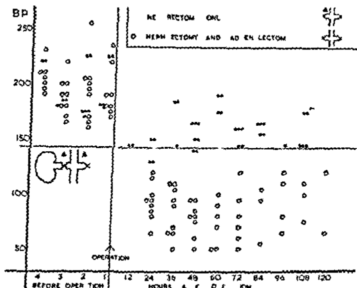


FIG 2 (Floyer) Comparison of the effect of total nephrectomy and of total nephrectomy and adrenalectomy in rats with chronic renal hypertension (Clin Sci 1951 10 401)

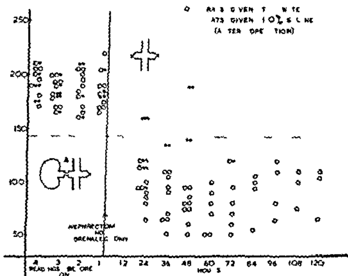


FIG 3 (Floyer) To show the effect of 1 per cent salt in drinking water on the blood pressure of rats with chronic renal hypertension after total nephrectomy and adrenalectomy (Clin Sci 1951 10 405)

LEFFRA In summarizing the evidence we cannot escape the fact that there are relationships and associations between sodium the adrenal glands and blood pressure regulation Furthermore there are differences between normotensives and hypertensives in their responses to sodium and to the administration of steroids We can for example produce hypertension in the adrenalectomized animal or in Addisonian man by the administration of cortisone even in the face of rigid sodium restriction whereas the hypertension of DCA in both animals and man appears to require at least some sodium The search for the common denominator is really the problem and its nature is not apparent at the moment To add to the confusion we have recently confirmed the studies of Freed Friedman and Rosenman that the reduction of potassium intake in hypertensive man is associated with small but significant drops in arterial tension at a time when there is significant sodium retention

I should like to raise a question should the attack on sodium adrenal relationships be shifted from water and sodium content in fluid compartments and in arterial walls in the direction of the effect of electrolytes on the reactivity of blood vessels For example there is the suggestive work of Fatt and Katz on the effect of changes in sodium on neuromuscular transmission and the production of acetylcholine Is it possible that we are missing an opportunity to approach the electrolyte and adrenal problem through some other way that has been overlooked to date?

that member usually develops hypertension Now if the adrenals in the already nephrectomized and hypertensive member are excised (second pair in figure) the blood pressure comes down not to hypotensive but to normal levels If the adrenals alone are removed from one member of a pair (third pair in figure) the blood pressure in this member continues within the normal range Thus the adrenalectomized parabiotic preparation is behaving differently in this respect from the single animal either as a result of the exchange of electrolytes or of the passage of adrenal factors across the vascular union from the intact member However whereas this exchange of electrolytes or passage of adrenal factors is sufficient to maintain a normal blood pressure in the adrenalectomized and nephrectomized member it is not sufficient to maintain hypertension For this to occur the animal's own adrenals appear necessary We conclude from this that an excess of some adrenal pressor factor is circulating in the blood of the nephrectomized animal and that this is an essential for hypertension to occur under these experimental conditions This state of affairs could come about either as a result of the adrenal functioning at a higher level than normal in the nephrectomized animal or as a result of the failure of inactivation by the kidney of adrenal pressor factors which are being produced at the normal rate We have no definite evidence either way as between these two possibilities

PICKERING But Dr Grollman has evidence that he can produce hypertension in the nephrectomized dog with adrenals absent which suggests that it is possible to produce it with no adrenal secretion At any rate in the dog if there is a pressor substance which is being manufactured and which is being destroyed by the kidney this experiment implies that the origin of the substance is not in the adrenals

FLOYER I was able to produce hypertension after nephrectomy and adrenalectomy only by giving salt when water alone is given hypertension does not occur I believe that Dr Grollman mentioned in his paper that his dogs which developed hypertension after nephrectomy and adrenalectomy were in fact taking up sodium from the dialysing fluid If this was the case those dogs were in the same position as my rats after nephrectomy and adrenalectomy taking in salt but not excreting it

GROLLMAN No because they are not allowed to take up salt but merely to maintain their normal sodium level Adrenalectomy is a procedure which induces abnormal effects which are superimposed upon the already existent conditions imposed by hypertension Hypotension reflects a severe degree of adrenal cortical insufficiency the presence of a normal blood pressure level merely indicates that the degree of insufficiency is not profound In patients suffering from Addison's disease as well as a moderate degree of hypertension the blood pressure may be normal but will rise when the patient receives adequate replacement therapy for his adrenal insufficiency I believe that essentially the same condition is present in your experimental animals

WILSON Could I ask Dr Perera about hypertension produced by different steroids?

salt—for the most part firstly 1 g per day and in a further course 2 g and 3 g per day unknown to the patient

It has to be pointed out that such an experiment can only be considered a reliable test if it has been made sure by daily analysis of urine that there really has been no greater intake of salt than 1 g per day with the food during the period of salt restriction

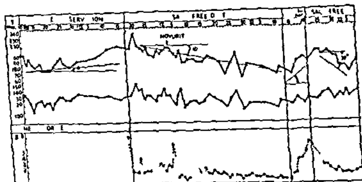


FIG 1 During the thirty five-day period of pre-observation, the patient had normal food with a salt intake of approximately 10 g per day During this time the blood pressure remained unchanged though towards the end of this period it seemed to rise a little In contrast to this the blood pressure decreased promptly and continuously with the beginning of salt restriction After seven weeks of salt free diet the food was changed by adding 3 g of salt daily Immediately the blood pressure rose again A period of only seven days caused a significant change in blood pressure During a second period of salt restriction the effect was partially reproducible The chloride content of the urine was measured daily during the salt free and low salt diet

Where the blood pressure values took a level course the therapeutic evaluation was done by a comparison of the mean pressure values However if there was a steady or sometimes a small decrease in blood pressure during the first period which implied an increase in the lowering effect during the time of salt restriction we *had to* compare the slopes of the two lines The statistical management had to be done here with the aid of mathematical definition of the degree

THE IMPORTANCE OF SODIUM CHLORIDE AND ITS IONS IN THE PRODUCTION AND THE TREATMENT OF HYPERTENSION

P MARTINI and K KAISER

In the treatment of essential hypertension it has become a question of the utmost importance to succeed in reducing hypertensive blood pressure This point is clearly brought out in the investigations of Pickering (1952) and Zollinger (1950), in addition to what we have heard in the past few days

I want to deal with the radical reduction of sodium chloride in the food, as it was first proposed by Allen thirty three years ago and with the relative importance of its ions and of the molecule

We tested this treatment seventeen years ago, and I am able to submit statistically significant results of its effectiveness I shall demonstrate our methods of evaluation with the aid of some illustrations In all diagrams there is a period number I the period of pre observation During this time nothing was changed in the habits of the patients except taking them into hospital and keeping them under observation there If some decrease of blood pressure values occurred this time of pre observation had to be made long enough to make sure there was no essential alteration in the further course of blood pressure That means that a well defined level or direction of systolic and diastolic pressures had to be ascertained in order to judge the further effects

During the second period once again only a single factor was allowed to be changed That was the restriction of salt Again, this period had to be prolonged until either the blood pressure had reached a constant horizontal plateau or its direction of change had become continuous

In a third period the period of post observation there was a change once again in a single factor the administration of

(1949) at hearing that many authors denied the beneficial effect of his low salt diet

In analysing the reasons for this difference in results we have found that most of the publications denying the effect give no guarantee that there is a real difference in the various periods of observation In many cases we see that the periods have no uniformity except in the management of salt intake Mostly we find a so called period of salt restriction but the intention to keep the intake of salt lower than 1 g per day has not in fact been realized and in reality there has been a higher intake In only a very few of these publications which give an unfavourable judgement on Allen s diet have daily routine salt controls been done This applies especially to the numerous publications which try to compare the results of surgical treatment of hypertension with those of treatment with salt restriction We found no guarantee that the diet had been applied properly

Another factor causing incorrect results is that patients with a low benign hypertension demonstrate a marked decrease in blood pressure under the influence of hospitaliza tion and rest In these patients this effect may be so complete before the beginning of salt restriction that no further statistically significant decrease can be expected Other authors had so selected their patients that nearly all of them had very advanced degrees of hypertension That also is one sided

Certainly we too have patients where the blood pressure refuses to fall under the treatment of salt restriction in these we have to accept a failure of the diet Apart from these cases I think we may point out that in most cases the failure of Allen s diet is only a delusion Knowingly or not the patients fail to keep to their strict diet This may be excusable as long as they really cannot follow the directions There are professions and situations where the difficulties in obeying the dietary regime are overwhelming But otherwise it seems foolish to stop the diet because the patient cannot stand it Sometimes the doctors are to blame for this as they them selves do not really believe in the value of Allen s diet and so

of inclination, comparing the coefficients of regression of the two periods (Martini, 1938, 1953) This gave a good security against errors and may be considered as proof, especially when the effect could be reproduced in quite a number of patients

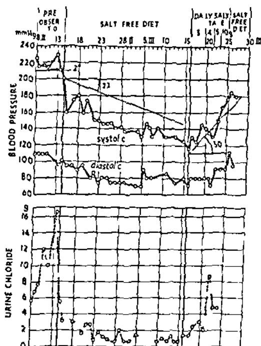


FIG 2 The diagram shows clearly the difference between blood pressure values during the pre and post observation periods on salt containing food compared with the period of salt restriction The correlation between blood pressure and the daily urinary chloride values should be noted (The diagram originates from 1937 so that only urine chloride was estimated)

We have seen that by the application of the strictest methods to our tests, there was a decrease in blood pressure in approximately three quarters of our patients following radical restriction of sodium chloride This decrease did not bring the blood pressure down to normal in many cases but we were able to lower it to relatively harmless values Since that time, I have been well able to understand Allen s anger

40 4 per cent chloride) up to a daily amount of 12 g There was no elevation of blood pressure In a third period we added instead of the chloride containing substitute a sodium containing one (containing 43 2 per cent sodium) up to 12 g per day Again the blood pressure remained at its low level After that we administered in a fourth period sodium chloride (12 g per day) and the blood pressure rose to a significant degree We ourselves are however not convinced of the absolute reliability of this experiment as the daily amount of added sodium during salt administration exceeded the sodium intake in the period when sodium bicarbonate was given

The method of investigation of another patient is demonstrated in Fig 3 which shows the administration of sodium

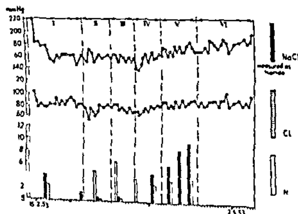


FIG 3 During salt restriction (I) the blood pressure drops to almost normal levels within five days and then remains horizontal Neither the addition of sodium alone (as "Curtasal" 12 g daily) (II) nor of chloride alone (as "Titrosina" 1. g daily) (III) raises the blood pressure When both sodium and chloride are added together (IV) as a mixture of Curtasal and "Titrosina (1 g daily) the blood pressure rises. This also happens when salt (V) is added in equivalent doses The column demonstrates the *output* of sodium and chloride in urine It is clear that *sodium* alone is not effective in hypertension The intake of sodium (controlled by the output in urine) in the earlier periods is larger than in the periods of combined sodium and chloride intake The amount of tubular reabsorption of sodium is not taken into consideration here but it is improbable that it would affect the conclusion in an essential manner

do not convincingly persuade the patient to hold out In fact, if an intelligent and dutiful patient carries out the salt free diet for the first eight successive weeks, from then on he will not miss salt much Then there are some patients who can take rather more than 1 g salt daily without risk of an increase in blood pressure

The cation exchange resins have been tolerated in the necessary doses in prolonged treatment by only a small number of our patients In these patients the results were very good, and it is possible that the further development of similar substances will bring valuable help to the performance of the salt free diet in hypertension

If it is possible to reduce the hypertensive blood pressure by the application of cation exchange resin—taking away the sodium ion from the contents of the stomach and intestines—it looks as if the sodium ion is the only factor in the sodium chloride molecule responsible for the hypertenson In 1938 I pleaded the thesis that the chloride ion gives the pressor reaction I came to that conclusion because at that time our substitutes for sodium chloride in the diet were *highly chloride free but contained approximately* 30–40 per cent sodium nevertheless they had no elevating effect on the blood pressure This has proved to be a logical error Later it was found out that there was in some cases of hyper tension (not in all cases) an elevated blood sodium level (Friedman *et al*, 1946 Knowlton *et al*, 1948 Laramore and Grollman 1950) It could be shown that on restriction of sodium the elevated blood pressure fell despite the administration of chloride containing substitutes On these facts the maintenance of the pressor reaction by sodium alone was proclaimed again

That neither the sodium free substitutes nor the chloride free substitutes elevate the blood pressure is a *contradiction* which we have tried to explain We have treated patients with hypertensive disease with salt restriction As usual, the blood pressure decreased In a second period we added to the salt free diet *a chloride containing* substitute (containing

DISCUSSION

CROLLMAN I would question the significance of the last slide on which Prof Martini has placed so much emphasis The patient to whom he referred appears to have been suffering from severe generalized arteriosclerosis rather than from hypertension since the diastolic pressure even following the administration of sodium chloride at the end of the experiment is relatively low

MARTINI Some of these patients had alterations in their vessels but it was not arteriosclerosis it was essential hypertension

CROLLMAN The rat with experimental hypertension is very sensitive to sodium depletion but will maintain its blood pressure at a hyper tensive level when administered sodium salts other than sodium chloride It was thus failure to appreciate the significance of the sodium ion as the important factor rather than sodium chloride *per se* which led to the failure of earlier advocates of salt restriction in the treatment of hyper tension Many patients placed on a salt free diet would continue to take sodium bicarbonate which exerts exactly the same effect as sodium chloride insofar as the blood pressure level is concerned The addition on the other hand of potassium chloride to a sodium depleted diet does not prevent the reduction in blood pressure There is a marked discrepancy between our experiments in animals and the experiments on the human reported by Prof Martini Despite the analogy which he presents I fail to see why sodium and chloride must be associated as a *single molecule* The chloride content of the body is relatively high and the administration of sodium bicarbonate would be *expected to exert* the same effect as sodium chloride under the conditions of his experiments since it would be converted to the chloride in the body

HILLMER We have treated quite a number of patients with hyper tension in the last three or four years with cationic exchange resins which remove sodium ion but not chloride ion Under these circumstances we get *excellent* depressions in pressure with the removal of sodium. When the patients are given salt substitutes containing potassium or ammonium chloride *no* elevation of pressure occurs It seems to us that it is the sodium ion and not the sodium chloride that is important

MARTINI But I believe not alone We must do more experiments We have done these experiments for half a year and it is always the same

PAGE Do you think that reduction to an excretory level of 1 g is *enough* Many of us think that about 200 mg is the upper limit to get a reasonable fall in *average blood* pressure in other words that 1 g is a little too high and that probably some of Allen s failures might have been due to the fact *that he did* nor require a lower sodium intake You get better results even *with the* 1 g intake than we do with the 200 mg I would say that at most 30 per cent of the patients get a really repeatable fall in arterial pressure on the *strict low* salt diet

MARTINI In the very early cases often it is not necessary to restrict salt intake but it depends on the other treatment and the other habits

as well as of chloride and later the combination of both ions in *one* patient We have tested this effect in a number of patients, but for different reasons we were not always able to carry through all test periods We have always seen the same effect

As the sodium chloride on entrance into the body dissolves practically completely into its two ions, we thought the two earlier views of the pressor effect of either the chloride ion alone or of the sodium ion alone in good agreement with our knowledge But as it has become obvious in our recent investigations that neither of these two ions by itself is able to increase blood pressure but only that both together can do it, the active agent must be the sodium chloride molecule This raises the question as to how this can be done and where the place could be where the two ions could join together for action Probably the membranes of the cells represent this critical site

Chemists have given us a comparison of the ions with dancing partners The one ion represents a lady the other a man *All partners are dancing in a ball room but everyone* for him or herself But every man is only allowed to leave the ball room together with his lady—they can leave only as a couple This image has no greater value than any other allegory, *but up to the present no chemist has been able to* give us a better interpretation

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of those of insurance companies have shown that the arterial pressure rises with age If one looks at the distribution of pressures in any particular decade one finds that the distribution is continuous not discontinuous And so when we divide up say at 150 systolic and 100 diastolic we are making a perfectly arbitrary division Now it is quite clear that some factor or factors are causing these differences of pressure in different individuals And it is quite possible that these factors may manifest themselves in other ways at a time when the arterial pressure is not particularly high. This may explain the facts to which Dr Perera drew attention in his paper He pointed out that in some subjects who had previously had hypertension and in others in which it was to be supposed that hypertension might subsequently develop some of the peculiar features of behaviour which he had observed in patients with hypertension could still be demonstrated

The second general remark I would like to make is that it is perfectly obvious that more than one mechanism can produce hypertension. Because we have demonstrated a mechanism in one particular set of circumstances it does not follow that an identical mechanism holds in an entirely different set of circumstances I think this is particularly relevant to the question of essential hypertension which is a condition defined on negative characteristics and there is of course no guarantee that it is in any way an entity indeed many of the observations made in this conference have suggested that it is not

I would like to proceed to discuss the extent to which we can identify the revelant mechanisms The first that we might consider are the proprioceptive reflexes arising from the carotid sinus and the arch of the aorta There is a good deal of evidence that these are in fact present in patients with hypertension. In the first place there is a certain amount of constancy of arterial pressure from day to day and when you reduce the arterial pressure by venesection it comes back fairly quickly and there is the usual blood dilution Not only that but the blood pressure is maintained during changes in posture We would like to know what is the frequency of the impulses ascending the carotid sinus and depressor nerves Are they more frequent than in subjects with lower pressures as you might expect if there was no change in the receptor mechanism are they less frequent as might be the case if some alteration in this mechanism were the primary cause of the rise in pressure or are they just the same as they are in patients with lower pressures? I don t think we have seen any clear way in which these questions can be answered but I am sure they are important questions and perhaps someone who leaves this conference will have a bright idea

The second problem is that of the neurogenic component Clifford Wilson and I some years ago thought we had answered this to our own satisfaction when it was demonstrated that when vasoconstrictor sympathetic tone was removed from the forearm and the hand the blood flow in hypertensive patients was no higher than it was in normal subjects So it looked as though the abnormal vasoconstriction was still present The same appears to be true of the brain and of the kidney It would also seem to follow that if hypertension persists after removal

of the patient In many cases it is not necessary to do anything for instance in people leading quiet lives but if you have a man in a nervous position the whole of his life it is often necessary to make a reduction of salt In the earlier years of this treatment all other supplements of salt were rich in sodium

PAGE Except the one that was rich in lithium A very enterprising outfit in the United States decided that *lithium chloride* would be a good salt substitute and so it was It tasted very much like sodium chloride The only difficulty was that as you reduced the sodium in the diet and substituted lithium the lithium began to go into the tissue *and the patients to go into coma*

MARTINI We had a coma in the last month using cation exchange resin a potassium coma

HELMER A most important consideration in treating patients with *cation exchange resins is to prevent potassium deficiency* When the sodium available to the resins is reduced considerable potassium is taken up on the resin To prevent potassium deficiency we give as much as 90–120 milliequivalents of potassium per day combined with metabolizable cations such as acetate citrate and bicarbonate between *resin dosages One of the best indications of impending potassium* deficiency is a decrease in the urine concentration of potassium This occurs about seven to ten days before a decrease *in plasma potassium* becomes evident

PAGE Do you use the electrocardiogram?

HELMER This is much better than electrocardiographic evidence *The decrease in potassium in the urine happens about ten days before* E C G changes occur

PAGE More expensive though!

* * * * *

PICKERING I am sure that you would like me to start by expressing the view of all of us namely that this has been a most enjoyable and instructive conference That has happened thanks firstly to the Ciba Foundation the Director and his Staff and secondly to the excellence of the company which we have enjoyed The papers have all been very clearly presented and they have been fully discussed and I m sure you don t expect me to comment on them individually I thought I might make one or two comments in a general way and ask a few questions

First on the definition of hypertension Dr Grollman has been of great value to this conference in producing some rather provocative statements I am afraid I didn t take down in detail his definition of hypertension—I am looking forward to reading that in print in the future book—but it does seem to me that the only way we can define hypertension is on the blood pressure recognizing that it is an arbitrary definition *All the surveys on population samples* with the exception

romantic career which Dr Helmer described to us yesterday What bothers me about this substance is that it is only demonstrable in the nephrectomized animal It bothered Govaerts too and I hope that Dr Helmer will inject not only 2 ml but large numbers of millilitres of the serum from his dead cats into a normal animal or else as Dr Floyer suggested into an animal with its renal artery clipped because if you can t demonstrate its action under those circumstances it is very difficult to see that it has anything to do with renal hypertension Also if its sole source is the kidney it is a little difficult to see that it has anything to do with the hypertension following nephrectomy

VDM and VTM have made only a temporary appearance at this meeting and I shall not disturb their rest Renin is under a cloud Govaerts very rightly criticized previous methods of obtaining the blood from hypertensive animals He got suggestive results with improved methods of obtaining blood It would be nice to see if using perhaps the method of catheterization of the renal vein or of the vena cava but with other methods of assay which might detect smaller quantities some kind of final answer could be obtained I must admit to having a certain prejudice in favour of renin It always seems to me that when one can produce hypertension by interfering with the kidneys and when in the kidney you have this very strongly acting and interesting substance and when you can produce hypertension in the totally sympathectomized dog that it is natural to suppose that renin might be concerned

Then there are the corticosteroids and here the discussion is so recent that I have not had time to think very much about them But it is quite clear that all the time the methods of assay of these substances are improving and that one substance after another is being identified In the next few years we shall get a clearer conception as to what part if any which corticosteroid if any plays in the development of particular kinds of hypertension

There has also been the question of alteration in electrolytes and extracellular fluid volume Here it seems to me that one of the important points is that to which Dr Ledingham drew our attention namely the distinction between what is intracellular and what is extracellular in the piece of muscle or other tissue in which measurements are made It does appear that at any rate this problem is beginning to yield to experimental method Perhaps at a subsequent meeting we may have a clearer answer to this problem

Next there is the problem of hypertension following nephrectomy I don t know what this is going to have to do with the hypertension that we see in man with kidneys but clearly it is a very interesting phenomenon and the answer to the mechanism may shed a new light on the whole problem of hypertension and may lead to completely new outlooks on the mechanism controlling blood pressure I do hope that Dr Grollman and those others interested in this problem will be able to tell us the answer before very long

Finally you may allow me to make a few comments on the nature of the malignant phase which is so important in the clinical management

of the whole sympathetic chain on both sides then that hypertension is probably not neurogenic although it is of course to be noted that there are no controls Nevertheless there may be some cases in which there is an abnormal neurogenic component Perhaps those are patients in whom the arterial pressure shows abnormally large swings during the course of the twenty four hours There may be other features which Page described in his diencephalic syndrome How can we identify the size of the neurogenic component? Page has suggested that it may be identified by the pattern of response to tetraethyl ammonium compounds Paton suggested three tests in which hexamethonium might be used Perhaps at our next meeting we may know the answers and we may know that it is possible to identify beyond any doubt certain patients in whom the unusually high pressure is due to neurogenic factors

Proceeding to humoral factors I would just like to say how little we really understand of the functions of these substances The thing that impresses me most is Heymans's totally sympathectomized dog which he mentioned quite briefly but which could fight quite successfully with dogs containing their whole complement of sympathetic nerves How does that dog regulate the pattern of its circulation which almost certainly must be done through humoral factors? When we have that depth of ignorance it is not surprising that we have not got very far in hypertension Adrenaline and noradrenaline are quite clearly responsible for the paroxysms of hypertension in phaeochromocytoma The changes during the paroxysms are exactly the same as the changes which you get when you infuse these substances into the circulation There is an increase in excretion of these substances in the period in which the paroxysm takes place and paroxysms cease when you remove the tumour At one time I had the very comfortable feeling that I did understand at least one kind of hypertension That comfortable feeling has rather disappeared during the course of this meeting in which it has been demonstrated that the hypertension can go on even though the excretion of noradrenaline is normal and even though the hand blood flow has returned to normal I also had the comfortable feeling that noradrenaline and adrenaline had nothing whatever to do with essential hypertension That persisted till yesterday when von Euler showed those figures for the excretion of noradrenaline Perhaps I can comfort myself that that is probably true for most of the patients with essential hypertension but there does seem to be a group in which it is perfectly possible that these substances are concerned Whether they are arising from the adrenal or from the arterial wall as Prof Jiménez Díaz suggests is a point that may eventually become clear

5-Hydroxytryptamine I think is a by product of research on hypertension It was probably because Irvine Page was interested in hypertension that he got on to it Of course it would have happened anyhow because Prof Erspamer was on to it from a different point of view But as far as we can judge it doesn't seem to have much to do with hypertension though it may have something to do with the control of renal function and perhaps in keeping us sane

Then there is that long acting pressor substance with the most

on the work that Clifford Wilson and I did many years ago on these arterial lesions in rabbits and the strongest evidence in its favour is provided by the beautiful experiments of Wilson and Byrom in which the lesions developed in the unclamped kidney but not in the clamped one as Dr Floyer has shown us again here

of hypertension The hypothesis that this phase is a simple consequence of the intensity of the hypertension and of the level of arterial pressure has come under a good deal of criticism at this meeting I think I ought to say that this hypothesis was deliberately oversimplified I never thought in my own mind that you could draw a line of the arterial pressure and say that above this the hypertension was going into a malignant phase and below it it was not I had always thought that if you took the two populations those in the malignant phase and those not they would have different means even though there was a good deal of overlap in their distribution I also thought that in a given individual the onset of the malignant phase would coincide with an increase in pressure The fact that it is possible to demonstrate that the malignant phase can be reversed to the benign under circumstances in which the arterial pressure falls seemed to me to be strong evidence for this hypothesis There are two main criticisms that have been made The first depended on the nature of the disturbance causing the peculiar retinal changes which I had attributed to raised intracranial pressure It has been pointed out that the intracranial pressure is not always raised It was raised in 12 out of 13 of my cases with albuminuric retinitis and in 19 out of 20 of the cases of Shelburne Blaine and O Hare s series in which papillœdema occurred But those figures are not very different from the figures on cerebral tumour In Ayer s series there were 42 patients with C S F pressures over 250 mm water and of those 39 had papillœdema and three did not Of 19 patients with pressures below 250 mm six had papillœdema and 13 did not How one accounts for the exceptions I don t know Some of them may be erroneous measurements I have observed that when my house physicians take the pressure they quite often let so much fluid out of the lumbar puncture needle before the manometer is connected up that the pressure is considerably reduced But I am sure that is not the only reason because I have also had the experience of recording lower pressures in patients with papillœdema and hypertensive disease when no loss of fluid has taken place The only thing I would like to say of this evidence is that its weight is of about the same order as the evidence that papillœdema in brain tumour is due to raised intracranial pressure

Perera s three cases and McMichael s one where the retinitis developed when the arterial pressure was low I just can t explain Sometimes one sees a retinitis which is very much like that of malignant hypertension from other causes I have seen it for example in patients with severe hæmatemesis and I have also seen it in acute disseminated lupus erythematosus But as I see it the function of a hypothesis is to stimulate inquiry and a hypothesis stands or falls by the facts and I respect the facts I am not quite sure what to put in its place McMichael expressed the opinion yesterday that the characteristic feature of the malignant phase was some kind of vasculitis My difficulty is that I don t know what he means by vasculitis it merely seems to be another name At any rate I cannot think of any method for finding out whether that explanation is right or not I might perhaps remind you that the hypothesis of the cause of the malignant phase is very largely dependent

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